THE
AMERICAN NATURALIST

VOL. LXIII *November-December, 1929* No. 689

THE FIRST CYTOLOGICAL DEMONSTRATION OF A TRANSLOCATION IN DROSOPHILA

PROFESSOR H. J. MULLER

UNIVERSITY OF TEXAS

IN 1926 C. Stern published an important article (16) describing a case in which the normal allelomorph of bobbed bristles, which is ordinarily present in the Y chromosome, behaved as if completely linked with (or substituted for) the gene for bobbed itself, which lies at the "inner" end of the genetic map of the X chromosome, and he presented cytological figures to show that "*an dem inneren Ende des X-Chromosoms ein Y-Chromosom angeheftet war.*" Here, then, there seemed to be a case of attached X and Y, paralleling the cases of two attached X's previously discovered by L. V. Morgan (8), by Anderson (1) and by Sturtevant (10). Careful examination of the figures indicated, however, as was mentioned in the course of a legend to them, that not the entire Y, but only the longer arm of it, had become attached, and later in the course of a more general and popular paper (17) published in 1927, Stern brought this point into relief and set against it the genetic finding that, unlike a complete Y, this attached Y did not suffice to prevent sterility in a male (though it did suffice to do so when in company with another incomplete Y, cytologically seen to lack a different section). Thus genetic as well as cytological evidence was here given, proving that there had been chromosome breakage followed by attachment of one of the pieces to a different chromosome.

Although the recipient chromosome in this case, the X, is in one sense homologous to the Y, and to this extent the

finding does not precisely fit the definition of a translocation once offered by the present author (breakage, with reattachment of a fragment to a non-homologous chromosome), nevertheless, it will be seen that the phenomenon described by Stern is essentially the same as that of a translocation. The first translocation, found by Bridges (3) in 1918, was too small to furnish definite cytological evidence of its existence by means of the methods then known, though Bridges has recently (1929) reported positive results on it (11). Stern's case thus was the first in Drosophila in which both genetic and cytological evidence of a translocation was combined, and it should have been referred to as such in the present author's discussions of translocations. Unfortunately, the author must plead guilty to having omitted citation of this case, owing to his having overlooked both the above-mentioned statement in the legend in the 1926 paper, and also the fact that additional important information concerning this case was given in the course of the more general paper of 1927 (following a discussion of well-known cases of other kinds—non-disjunction, gyrandomorphs, etc.). These oversights led to his making such statements as the following concerning translocations: "only one example of which (translocations) seems to have been definitely established in previous Drosophila work, by Bridges in 1918" (13); and (in reference to an X-ray translocation demonstrated in 1927) "this was the first microscopical demonstration in Drosophila of a chromosome breakage known to be associated with a genetically determined translocation" (14). It is to rectify these errors that the present article is written.

In discussing the combination X-Y chromosome in his 1926 paper, Stern very clearly brings out the bearing of the case on the theory of linear linkage. For the genetic fact that non-bobbed (representing the Y) was linked to bobbed, which is at the extreme "inner" end of the genetic map, agreed with the cytological fact that the Y (or piece of it) was attached to the inner end of the

actual X chromosome; bobbed was therefore in the precise physical position that the linkage map represented it to be in. The evidence from this case thus agreed completely with that previously found in the cases of attached X's studied by L. V. Morgan (8, 9), Anderson (1) and Sturtevant (10). Here it had been originally deduced by Anderson from the frequency of crossing over between the point of union of the X's and the loci of various known genes, that this point of union was at one end of the genetic map, at or very near the locus of bobbed. The fact that it was the end of the genetic map containing the bobbed locus which tended to remain heterozygous in eggs that received two X's through primary non-disjunction or parental triploidy (4) had further indicated that this was the end at which the spindle fiber was normally attached (the "inner" end). The cytological evidence, on its side, had harmonized with all this genetic evidence by demonstrating that the X's were indeed attached at their ends, and, in fact, at their "inner" (spindle fiber) ends, and it had thus afforded a direct proof of the physical validity of the map, in so far as the position of bobbed was concerned.

The verification of the position of bobbed, both by the cases of the X-X and of the X-Y combination chromosomes had thus made available the first direct cytological confirmation of the validity of the position of a gene as shown in a linkage map, and this evidence should be recognized as antedating ours, which dealt with the arrangements of various other genes, as studied through translocations and deletions produced by X-rays. In the X-Y case, moreover, not only was this evidence merely made available, but it had been overtly recognized and its significance for the theory of linear linkage had been specifically pointed out by Stern. Unfortunately, the investigation of this case could not be carried further, so as to show that all loci in the Y lying on one side of a given point in the linkage map (the breakage point) had become transferred, since there can be no linkage map of

the Y, due to the fact that it never undergoes crossing over. The verification of linkage maps had, therefore, to be confined to a determination of the position of the attachment point on the recipient chromosome (*i.e.*, the bobbed locus on the X). Likewise, in the case of the attached X's, there was no breakage point which could be (or, at least, which has been) dealt with genetically, only an attachment point (close to the bobbed locus).

In many of the cases of translocation and deletion caused by X-rays, on the other hand, the breakage point as well as the attachment point can be dealt with genetically, and it can be shown that a group of genes forming one coherent block on the linkage map has become transferred or lost. This, in connection with the cytological evidence of transfer or loss of a section of the corresponding chromosome, shows that the genes lying together on the map are physically together, *i.e.*, that the map sequence is correct so far as the relations of the genes to right or left of all points of breakage thus far investigated are concerned. The principle here involved is, of course, similar to that involved in verifying the attachment points, but the evidence is more varied and abundant since breakage points may be anywhere, whereas attachment points are usually only terminal.

It was this evidence regarding breakages, then, which was new in the X-ray work, and it was this which the author particularly had in mind when emphasizing the bearing of these findings on the arrangement of the genes. Even here, to be sure, there had been plenty of antecedent genetic evidence of a similar nature—Bridges's and Mohr's "deficiencies" (2, 5, 6, 7), Bridges's translocation (3) and Stern's cases of elimination of portions of chromosome III in somatic tissue (19)—but it happened in all these cases that, for one reason or another, cytological evidence of a breakage, transfer or loss of chromatin could not be obtained, and therefore the direct test of the correctness of the interpretation offered was lacking.

The use of X-rays gets around this difficulty simply by providing such an abundance of cases that, among them all, some of a suitable nature both for a genetic and a parallel cytological analysis are to be found. In this way it has now been possible to demonstrate, by a combination of genetic and cytological evidences, not only that bobbed lies at the "inner" end of the X, but (for example) that at the "outer" end lie each of the following genes in the order named: yellow, scute, broad, prune, white and facet, just as the linkage map shows them, and that the other genes, shown beyond them, actually do lie beyond; that star lies near one end of chromosome II; that ru and h lie to one side of a point on the other side of which lie st p ss e (as on the map of chromosome III); that st is between ru h and p ss e (as on the map); that p ss and sr are in a block lying between st and e ro ca (as on the map), etc. In view of all these, and other, correspondences found in the X-ray work between the actual and the linkage-map arrangements of genes, the truth of the latter can no longer be doubted. This evidence was arrived at independently of the evidence concerning the position of bobbed, which, unfortunately, was not thought of by the writer in relation to the translocation work. It is for this reason, however, all the more desirable that the priority of the work on the position of bobbed be pointed out now, and the writer wishes to acknowledge his indebtedness to Dr. Stern for having redirected his attention to that work.

BIBLIOGRAPHY

1. Anderson, E. G.
1925. "Crossing-over in a Case of Attached X-chromosomes in *Drosophila melanogaster*." *Genetics*, 10: 403–417.
2. Bridges, C. B.
1917. "Deficiency." *Genetics*, 2: 445–465.
3. Bridges, C. B.
1923. "The Translocation of a Section of Chromosome II upon Chromosome III in Drosophila." (Abstr.) *Anat. Rec.*, 24: 426.
4. Bridges, C. B., and E. G. Anderson.
1925. "Crossing-over in the X-chromosome of Triploid Females of *Drosophila melanogaster*." *Genetics*, 10: 418–441.

5. Mohr, O. L.
1919. "Character Changes Caused by Mutation of an Entire Region of a Chromosome in Drosophila." *Genetics,* 4: 275–282.
6. Mohr, O. L.
1923. "Das Deficiency-Phänomen bei *Drosophila melanogaster.*" *Zeits. ind. Abst. u. Vererb.,* 30: 279–283.
7. Mohr, O. L.
1923. "A Genetic and Cytological Analysis of a Section Deficiency Involving Four Units of the X-chromosome in *Drosophila melanogaster.*" *Zeits. ind. Abst. u. Vererb.,* 32: 108–232.
8. Morgan, L. V.
1922. "Non-criss-cross Inheritance in *Drosophila melanogaster.*" *Biol. Bull.,* 62: 267–274.
9. Morgan, L. V.
1925. "Polyploidy in *Drosophila melanogaster* with Two Attached X-chromosomes." *Genetics,* 10: 148–178.
10. Morgan, T. H., C. B. Bridges and A. H. Sturtevant.
1925. "The Genetics of Drosophila." Bibliographia Genetica, II, 262 pp.
11. Morgan, T. H., A. H. Sturtevant and C. B. Bridges.
1928. "The Constitution of the Germinal Material in Relation to Heredity." Carnegie Inst. Yrbk., 27: 330–335.
12. Muller, H. J.
1927. "Artificial Transmutation of the Gene." *Science,* 66: 84–87.
13. Muller, H. J.
1928. "The Production of Mutations by X-rays." *Proc. Nat. Acad. Sc.,* 14: 714–726.
14. Muller, H. J., and T. S. Painter.
1929. "The Cytological Expression of Changes in Gene Alignment Produced by X-rays in Drosophila." AMER. NAT., 63: 193–200.
15. Painter, T. S., and H. J. Muller.
1929. "The Parallel Cytology and Genetics of Induced Translocations and Deletions in Drosophila." *Jour. Hered.,* 20: 287–298.
16. Stern, C.
1926. "Eine neue Chromosomaberration von *Drosophila melanogaster* und ihre Bedeutung für die Theorie der linearen Anordnung der Gene." *Biol. Zentr.,* 46: 505–508.
17. Stern, C.
1927. "Die genetische Analyse der Chromosomen." *Naturwis. Jr.,* 15, Hft. 22: 465–473.
18. Stern, C.
1927. "Über Chromosomenelimination bei der Taufliege." *Naturwis. Jr.,* 15, Hft. 36: 740–746.
19. Stern, C.
1927. "Elimination von Autosomenteilen bei *Drosophila melanogaster.*" *Zeits. ind. Abst. u. Vererb.,* Sup. Bd. II: 1403–1404.
20. Stern, C.
1928. "Fortschritte der Chromosome-Theorie der Vererbung." *Ergeb. d. Biol.,* 4: 205–359.

SEX DETERMINATION IN SCIARA[1]

DR. CHAS. W. METZ

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION OF WASHINGTON
COLD SPRING HARBOR, N. Y.

THE problem of sex determination in Sciara has presented many puzzling features due to the presence of a series of peculiarities, both chromosomal and genetic, which have proven difficult to harmonize on any simple scheme. These peculiarities have been considered individually in recent papers dealing with successive steps in the investigation (see appended bibliography) but, up to the present time, the evidence has not been complete enough to bring the different features into relationship with one another. With the completion of studies now in press, however, the main facts become related sufficiently, it is believed, to reveal the general nature of the processes involved. The present paper, therefore, is an attempt to bring the evidence together and to indicate some of the conclusions which it seems to require.

In doing this, allowance is made for the fact that the processes of oogenesis and fertilization have not yet been studied, that various known aberrant characteristics still remain to be investigated and explained and that in some respects the relationships between sex and chromosome behavior have become increasingly intricate and puzzling as the investigation has progressed. The latter features, however, appear to be concerned mainly with the influence of sex on chromosome behavior, rather than the reverse, and are, therefore, taken into account merely in so far as they seem to show any possibility of altering the interpretations presented.

TERMINOLOGY

The term "sex determination" is used in its broad sense, as will appear below. Since at least two appar-

[1] This investigation has been aided by a grant from the National Research Council, Committee for Research in Problems of Sex.

ently distinct sets of phenomena are involved in sex determination here, a distinction is made between what may be called the "determination of the sex of the progeny as a whole"[2] and the "determination of the sex of the individual." For want of better terms, the former may be referred to as "sex of progeny" and the latter as "sex of individual." The word "progeny" refers to all the offspring of a pair, or of a single female.

RÉSUMÉ OF EVIDENCE

The cytological findings are based upon a careful study of five species and a cursory examination of several others within the genus. The breeding work has involved all of these species to some extent, since it is necessary to rear the material used for cytological study, but for the genetic experiments *S. coprophila* Lint. and *S. impatiens* Joh. have been used primarily. Where not otherwise indicated, the facts, both cytological and genetic, apply to *S. coprophila.*

Chromosome number and behavior. (1) The males normally possess two more chromosomes than the females, the diploid numbers being ten and eight respectively (Metz, '26).

(2) The two extra chromosomes in the males are much larger than the others and are readily identified. They are referred to as the "sex limited" chromosomes. They do not pair with, or associate intimately with, any of the other chromosomes and they have no counterparts in the female. In some species the two differ from one another in shape and may be identified individually.

(3) These "sex limited" chromosomes remain condensed during the growth period of the primary spermatocyte in the manner characteristic of sex chromosomes.

(4) During the aberrant, unequal, monocentric reduction division in the primary spermatocyte, both "sex

[2] For the sake of brevity this was previously referred to as "sex-ratio determination." Since it is not actually the sex ratio, but rather the sex of the entire progeny, which is involved, the earlier term has been abandoned.

limited" chromosomes and one member of each pair of "paired" chromosomes pass to the single pole and remain in the cell (which becomes the second spermatocyte). The other four chromosomes are cast off in a bud which degenerates.

(5) At the second spermatocyte division (which is likewise unequal, but has a bipolar spindle) all the chromosomes divide and all send daughter halves to both poles, except one of the "paired" chromosomes. Both halves of this chromosome pass precociously to the pole which is to remain in the cell (spermatid). The other pole is budded off and degenerates.

(6) Each spermatid thus receives seven chromosomes, including both "sex limited" chromosomes and both halves of the "precocious" chromosome.

It is not known that any relation exists between the "precocious" chromosome and sex, but its behavior is noted here because the significance of its peculiarities is undetermined.

(7) Oogenesis has not yet been studied. The only evidence bearing on chromosome behavior here is genetic and it reveals no constant peculiarities.

Genetic behavior. (1) No parthenogenesis (or gynogenesis) occurs. All eggs must be fertilized in order to develop.

(2) In most of the species studied "unisexual" progenies are regularly obtained from pair matings.

(3) The sex of the progeny as a whole—*i.e.,* whether it is a female progeny or a male progeny—is determined by the genetic constitution of the mother.

(4) Female-producing females regularly give female-producing and male-producing daughters in equal numbers. In one line the two types of females are distinguishable by means of a mutant character, the gene for which appears to be completely linked with the differentiator responsible for "sex of progeny" (unpublished data).

(5) "Exceptional" males and "exceptional" females often appear in small numbers in progenies predomi-

nantly of the opposite sex, showing that the mothers are potentially capable of producing offspring of both sexes.

(6) "Exceptional" females from male progenies are all male-producers (twenty-eight tested).

(7) In three of the species normally giving "unisexual" progenies strains have been secured which give bisexual progenies (unpublished data).

(8) In *S. coprophila* (one of the species giving "unisexual" progenies) a mutant character has been studied which shows sex linked inheritance of the kind expected where the female is XX and the male XY in sex chromosome constitution.

(9) Individual males give both sons and daughters and hence are assumed to produce both X-bearing and Y-bearing sperms.

(10) Careful egg counts, compared with counts of mature flies developed from the same lots of eggs, show that the "unisexual" progenies are not caused by differential mortality of zygotes after the time the eggs are laid (data in press). Other studies show similarly that the "unisexual" progenies are not caused by a selective retention of fertilized eggs by the female; and they also indicate that no degeneration of fertilized eggs takes place in the female (data in press). From these observations it is concluded that differential mortality of zygotes is not the cause of "unisexual" progenies.

DETERMINATION OF THE SEX OF THE "PROGENY AS A WHOLE"

From the nature of the evidence it is convenient to consider first the "sex of the progeny." In doing this we are concerned only with conditions in those species which normally give "unisexual" progenies. Since the sex of the progeny as a whole is determined by the zygotic constitution of the mother and since female-producing females give female-producing and male-producing daughters in equal numbers, it is considered that the female-producing female is heterozygous for a pair of factors or chromosomes responsible for this behavior. The simple assumption that the genetic constitutions are

as follows fits the facts thus far known, including tests of "exceptional" females.

Female-producing female	*Aa*
Male-producing female	*aa*
Male	*aa*

On this basis, the factor or chromosome *A* would be confined to the female-producing female passing regularly from mother to daughter (half the daughters). Studies on a bisexual strain of *S. coprophila,* recently secured from laboratory stock, suggest that *A* and *a* represent more than single factors, but the evidence is not yet complete. Other evidence, mentioned above and likewise incomplete, indicates that *A* is in, or associated with, the X-chromosome. If it is a part of the X-chromosome, then it is apparent that there are two kinds of X-chromosomes here, X^A and X^a, the former (or the *A* portion of it) being limited to female-producing females. Thus instead of the two types of sex chromosome combinations noted in the following paragraph, there would be three—X^AX^a, X^aX^a and X^aY.

Determination of the "Sex of the Individual"

The determination of the sex of the individual fly is apparently dependent on the distribution of sex chromosomes of the XX-XY type, the female being XX and the male XY. The evidence here is entirely genetic (except as regards the presence of Y which is inferred from cytological data) but it appears, nevertheless, to be unequivocal. Since the earlier paper on this subject (Metz and Ullian, '29) was written, further tests have been made with the sex linked character there described and also with a second sex linked mutant character. These involve the bisexual strain mentioned above, as well as the unisexual strains, and show that although there are frequent deviations from the expected 1:1 ratio these are evidently due to secondary disturbances—probably disturbances interfering with random segregation in the female. The later tests also indicate that the peculiar

inheritance following the cross with the California race, described in the earlier paper, is due, as there suggested, to some genetic peculiarity of this particular race and has no bearing on the present question.

Perhaps in material presenting as many peculiarities as does Sciara, any conclusion should be held with some reserve at the present stage, but there is no other reason, in the writer's opinion, for qualification in this case. Consequently, the conclusion that chromosomes of the XX-XY type are present is used as the basis for the interpretation of sex determination outlined here.

On this basis, the sperm is considered as the sex "determining" agent due to its transmitting X or Y. Since a male may give both sons and daughters, apparently in equal numbers, it is concluded that he produces both kinds of sperms in equal numbers, and hence that segregation of X and Y occurs at random during spermatogenesis. The latter conclusion is of interest because segregation of the autosomes here, so far as known, is highly selective (Metz, '26, '28).

THE CAUSE OF "UNISEXUAL" PROGENIES

Obviously, if the above conclusions are correct, several phenomena remain to be explained. One of these is the production of "unisexual" progenies.

With both X-bearing and Y-bearing sperms produced by the male and received by the female, it is necessary to assume the action of some selective process to account for the regular elimination of one sex. This selection may act on the zygotes or on the gametes. The former would involve a regular process of differential mortality such as to eliminate the male zygotes from female-producing females and the female zygotes from male-producing females. It would necessarily have to occur after the fertilization of the eggs. The second alternative, on the other hand, would involve an elimination (inactivation) of sperms—Y-bearing in the one case and X-bearing in the other—and would occur at or before fertilization of the eggs.

The hypothesis of differential mortality is susceptible of experimental test, since the elimination of half the eggs or embryos, which it requires, should readily be observed by following the history of the eggs after fertilization. Such observations have been made (data in press) as indicated above, and they show no indication of differential mortality at any stage. The exact time at which the eggs are fertilized has not yet been determined, but since the entire history of the eggs has been followed the evidence seems conclusive, nevertheless.

From this we are led to assume that it is the sperms, rather than the zygotes, which are acted upon selectively. However, there is no available method of observing such a process directly at present and it need not be considered here further than to note that it might occur in the sperm receptacles of the female or in the eggs at the time of fertilization, and that, in either case, the result would be due to the zygotic constitution of the female.

Rôle of the "Sex Limited" Chromosomes

In the above scheme of sex determination the "sex limited" chromosomes play no part and at present their function, and their fate where females are produced, is unknown. That they have a vital function is indicated by their wide-spread presence in the genus and by their degree of constancy. That they are not "sex determining" in function, however, is indicated both by the evidence reviewed above and by the fact that one species apparently lacks them entirely (unpublished data). In the species studied most (cytologically) they show various irregularities in number, ranging from one to three in different individuals, which, together with their apparent absence in one species, indicates that their genetic make-up is probably not comparable with that of the other chromosomes, except perhaps the Y.

Interest attaches to the possible phylogenetic history of these chromosomes, but there is little evidence from which to draw inferences at present. Certain facts suggest the hypothesis, mentioned in earlier papers, that

they may at one time have been a true sex chromosome pair, and that now their sex "determining" function has been transferred to another pair, with a consequent loss of functional importance which has led to their elimination from one species and their aberrant behavior in others. The main justification for this hypothesis, at present, is the behavior of these chromosomes during the growth stages of the primary spermatocytes. Here they remain condensed in the manner so generally characteristic of true sex chromosomes in insects, and in a more marked fashion, apparently, than any of the other chromosomes.

The mechanism of elimination of the "sex limited" chromosomes required by their absence in females will be revealed, it is hoped, by a study of fertilization and early cleavage. The elimination may be effected by the sperm due to the presence of the X-chromosome in this class; or it may be effected by the egg at or after fertilization.

THE "PRECOCIOUS CHROMOSOME"

The "precocious" chromosome need be considered here only in connection with the possibility that it may be a sex chromosome—a possibility which might, perhaps, be suggested by its peculiar behavior. This behavior, however, does not agree with that expected of a sex chromosome on the basis of the genetic evidence outlined above. Since the cytological evidence indicates that there is no synapsis during spermatogenesis, the "precocious" chromosome, being in the second spermatocyte, is to be considered as one member of the original pair of chromosomes. And since both halves of this chromosome go into the spermatid together, they represent essentially a pair of identical chromosomes, which is presumably transmitted by the sperm.

Until it is ascertained what the corresponding chromosomes do in the maturation of the egg, it is of little value to speculate on the type of inheritance expected from such behavior. It is to be noted, however, that the sex

linked inheritance considered above does not call for the transmission of two identical sex chromosomes, either X or Y, by the sperm.

If in half the cases the precocious chromosome were to pass to the pole which remains in the spermatid, and in the other half were to pass to the pole which goes off in the bud, two types of sperms would result. But this would not avoid the other difficulties and, furthermore, it is opposed by the cytological evidence which, although difficult to make conclusive, indicates that the "precocious" chromosome always goes to the pole which is to remain in the spermatid. The evidence at present, therefore, points strongly toward the conclusion that the "precocious" chromosome is not concerned in sex determination.

Summary

(1) See summary of cytological and genetic data on pp. 488–490.

(2) On the basis of these data the following conclusions are drawn respecting sex determination.

(3) Sex determination (in the broad sense) involves two distinct series of phenomena: (a) determination of the sex of the progeny as a whole, (b) determination of the sex of the individual.

(4) Sex of the progeny is determined by the zygotic constitution of the female. Presumably female-producing females are *Aa*, male-producing females *aa* and males *aa* in constitution—*A* and *a* representing either chromosomes or genes.

(5) Sex of the individual is determined in the ordinary fashion by a pair of sex chromosomes (XX ♀, XY ♂).

(6) *A* and *a* are probably "in" the X-chromosome or associated with it.

(7) "Unisexual" progenies are apparently the result of a selective elimination (inactivation) of sperms, not of zygotes.

(8) The "precocious" chromosome is probably not a sex chromosome.

(9) The "sex limited" chromosomes are apparently not directly concerned in sex determination.

LITERATURE CITED

Metz, Charles W.
1925. "Chromosomes and Sex in Sciara." *Science*, n. s., 61: 212–214.

———.
1926. "Chromosome Studies on Sciara (Diptera) I: Differences Between the Chromosomes of the Two Sexes." AMER. NAT., 60: 42–56.

———.
1926. "An Apparent Case of Monocentric Mitosis in Sciara (Diptera)." *Science*, n. s., 63: 190–191.

———, M. S. Moses and Ellen N. Hoppe.
1926. "Chromosome Behavior in Sciara (Diptera) I: Chromosome Behavior in the Spermatocyte Divisions." *Zeitsch. f. induk. Abstammungs- u. Vererb.*, Bd. 42, Heft 4, 237–270.

———.
1926. "Genetic Evidence of a Selective Segregation of Chromosomes in Sciara (Diptera)." *Proc.* Nat. Acad. Sci., vol. 12, No. 12, 690–692.

——— and M. S. Moses.
1926. "Sex Determination in Sciara (Diptera)." *Anat. Rec.*, vol. 34, No. 3, 170.

———.
1927a. "Chromosome Behavior and Genetic Behavior in Sciara (Diptera). II: Genetic Evidence of Selective Segregation in *S. coprophila.*" *Zeitsch. f. induk. Abstammungs- u. Vererb.*, vol. 45, No. 3, 184–200.

———.
1927b. "Chromosome Behavior and Genetic Behavior in Sciara (Diptera) III: Absence of Parthenogenesis or Gynogenesis in *S. coprophila.*" *Ibid.*, vol. 45, No. 3, 200–201.

———.
1928. "Genetic Evidence of a Selective Segregation of Chromosomes in a Second Species of Sciara (Diptera)." *Proc.* Nat. Acad. Sci., vol. 14, No. 2, 140–141.

Moses, M. S., and C. W. Metz.
1928. "Evidence that the Female is Responsible for the Sex Ratio in Sciara." *Ibid.*, 14: 928–930.

Metz, Chas. W., and M. S. Moses.
1928. "Observations on Sex-Ratio Determination in Sciara." *Ibid.*, 14: 930–932.

Metz, Chas. W., and Silka S. Ullian.
1929. "Genetic Identification of the Sex Chromosomes in Sciara." *Ibid.*, 15: 82–85.

NEW EVIDENCE BEARING UPON THE PROBLEM OF THE CYTOLOGICAL BASIS FOR GENETICAL PECULIARITIES IN THE OENOTHERAS[1]

DR. RALPH E. CLELAND[2]
GOUCHER COLLEGE

AND

FR. OEHLKERS
DARMSTADT, GERMANY

THIS is a preliminary report of a joint cytological and genetical study of a number of species and species-hybrids of Oenothera, which was made in the hope of obtaining experimental evidence upon the interesting problem of linkage presented by this genus. The material was grown during the summers of 1927 and 1928 in the botanical garden at Tübingen, Germany, with support provided by the Notgemeinschaft der deutschen Wissenschaft. The cytological study was begun in the Botanical Institute at Tübingen, continued in the Botanical Institute at Jena and completed at Goucher College, Baltimore.

Our procedure was to grow during the first summer the F_1's of reciprocally made crosses involving seven species as parents, together with the parents themselves, making both cytological and genetical studies; and during the following summer to grow and analyze the F_2's and back-crosses, where these were not already sufficiently known from the literature. Thus a direct comparison was made between the cytological and genetical behavior of a large number of plants belonging to a considerable range of hybrid combinations. The chief responsibility for the cytological results falls upon the first author, who is also largely responsible for this preliminary account; the genetical results have been obtained by the second author.

[1] Read before the Joint Genetics Sections of the Botanical Society of America and the American Society of Zoologists, on December 27, 1928.

[2] Fellow of the John Simon Guggenheim Memorial Foundation, June, 1927, to September, 1928.

We shall first present in condensed form the chief cytological results of our study, followed by a brief review of the current theories as to the relation between chromosome behavior in meiosis and the formation of large complexes of linked genes in this genus. The genetical data will then be summarized, and briefly discussed in the light of the cytological facts.

Before presenting the cytological conclusions of our work, we may take time to state the essential features of meiosis, as found in most of the species of Oenothera. They consist (1) in the presence in diakinesis of a large circle or chain of chromosomes which are attached end to end, *the number of chromosomes involved in the chain being constant in each race;* (2) *in the retention of this circle intact throughout the heterotypic metaphase, and the final passage of adjacent chromosomes to opposite poles,* a fact which gives to the circle a regularly zigzag appearance just previous to the final separation of chromosomes in anaphase. For the first accounts of these phenomena, see Cleland, 1922, 1923, 1924a, b.

A summary of the chromosome arrangements, as determined by us for each of the seven species and twenty different hybrid combinations which were studied jointly in this work, is contained in Table I. The following are the cytological facts brought to light in the present investigation to which especial attention is called.

1. Six of the seven species used as parents belong to the group of so-called "complex-heterozygotes" (Renner) or permanently heterozygous species (all except *O. Hookeri*). It has been shown previously (Cleland, 1927) that in complex-heterozygotes, at least twelve and usually all fourteen of the univalent chromosomes are unpaired and united end to end to form circles or chains in diakinesis and the heterotypic metaphase. To the list of such species, whose chromosome configurations are already known, are added as a result of this study several new ones, all of which possess a circle of twelve or fourteen chromosomes in their pollen mother cells (see

TABLE I

CHROMOSOME CONFIGURATION OF SPECIES AND HYBRIDS STUDIED BY THE AUTHORS

Species or Cross	Complex combination	Chromosome configuration
Hookeri	ᵇHookeri. ᵇHookeri	7 pairs
grandiflora (de Vries)	acuens. truncans	⊙* of 14
Lamarckiana (de Vries)	velans. gaudens	⊙ of 12, 1 pair
Lamarckiana cruciata (Oehlkers)	velans. gaudens	⊙ of 12, 1 pair
strigosa	deprimens. stringens.	⊙ of 14
suaveolens and *suaveolens sulfurea*	flavens. albicans	⊙ of 12, 1 pair
Cockerélli	curtans. elongans	⊙ of 14
grandiflora × *Hookeri* and reciprocal	acuens. ᵇHookeri & recip.	2 ⊙s of 4, 3 pairs
	truncans. ᵇHookeri & recip.	⊙ of 14
grandiflora × *Lamarckiana* and reciprocal	acuens. velans & recip.	⊙ of 4, ⊙ of 6, 2 pairs
	acuens. gaudens & recip.	⊙ of 14
	truncans. velans & recip.	⊙ of 4, ⊙ of 10
	truncans. gaudens	⊙ of 4, ⊙ of 10
Lamarckiana cruciata × *strigosa* and recip.	velans. stringens	⊙ of 4, ⊙ of 6, 2 pairs
	gaudens, stringens	⊙ of 14
	deprimens. velans	⊙ of 10, 2 pairs
	deprimens. gaudens	⊙ of 10, 2 pairs
Lamarckiana × *suaveolens sulfurea* and reciprocal	velans, flavens & recip.	2 ⊙s of 4, 3 pairs
	gaudens. flavens & recip.	⊙ of 12, 1 pair
	albicans. velans	⊙ of 14
	albicans. gaudens	⊙ of 6, ⊙ of 8
suaveolens sulfurea × *strigosa* and recip.	flavens. stringens	⊙ of 4, 5 pairs
	albicans. stringens	⊙ of 12, 1 pair
	deprimens. flavens	⊙ of 12, 1 pair
suaveolens × *Cockerelli* and reciprocal	albicans. elongans	⊙ of 12, 1 pair
	flavens. elongans	⊙ of 8, 3 pairs
	curtans. flavens	⊙ of 12, 1 pair

* ⊙ = circle.

Table I). Thus additional evidence is presented in favor of the contention *that complex-heterozygotes, as opposed to other wild species, have as one of their chief characteristics the presence of at least twelve unpaired end-to-end chromosomes in diakinesis and the heterotypic metaphase.*

2. It has been found that hybrids are as constant from the standpoint of chromosome configuration as are the wild species. The rule is that individuals having the same complex-combination possess identical chromosome arrangements, which are found in all their pollen mother cells. Exceptions to this rule are apparently as rare in the case of hybrids as they are in wild species.

3. The chromosome configurations in the diakinesis of hybrids, while constant in each given form, show a wide variation in type in the various forms, ranging from configurations in which all the chromosomes are united end to end to form a large circle, to those in which there are several independent pairs (see Table I). In this respect, hybrids may differ strikingly from the wild species, which from this standpoint fall into two quite distinct classes—those with large circles, and those with no circles at all, but instead, perfect pairing in diakinesis. In a number of hybrids, two circles are present, an interesting feature found so far in only one wild species, *O. biennis*.

4. Hybrid combinations obtained reciprocally have in all cases shown the same chromosome configuration (see Table I).

5. The percentage of irregularity in the zigzag arrangement in metaphase ranges from almost zero in forms with the smallest circles to an average of from 30 to 35 per cent. in forms with a circle of fourteen. In respect to the percentage of irregularity, therefore, hybrids do not differ widely from the spontaneous species, in cases where their chromosome configurations are comparable.

In concluding this brief summary of cytological findings, especial attention may be drawn to the fact that

while a number of hybrids show chromosome configurations similar to those found in the permanently heterozygous species, a goodly proportion on the other hand have configurations in which the circles are small, and in which there are several independent pairs of chromosomes.

Now, the purpose of our investigation was to determine whether the presence or absence of large circles in meiosis has anything to do with determining the hereditary behavior of the individual or not. Largely due to the brilliant work of Professor Renner and his followers, it is now known that most species of the subgenus Onagra produce as a rule no more than two recognizable classes of gamete. In each individual these two types are almost, if not wholly, identical in genetic constitution with the sperm and the egg which united in the first place to form the individual. In other words, maternal and paternal gene complexes are passed on essentially intact from generation to generation, with little or no admixture of genes from the opposing complex. If we think of this situation in terms of linkage, we are forced to the conclusion that by far the greatest proportion, if not all, of the genes affecting visible characters in each species belong to a single linkage group.

Two theories relative to the physical basis underlying this interesting and unique behavior are before the scientific public for consideration. The first of these (see Cleland, 1923, 1924a, etc.; Oehlkers, 1926; Håkansson, 1926; Sheffield, 1927; Gates, 1928) claims that there is a fundamental connection between chromosome configuration and genetical behavior. The union of most of the genes into a single linkage group in the permanently heterozygous species in spite of the presence of fourteen chromosomes, and the presence of but two kinds of gene complex in the gametes, is considered to be due to the union of most or all of the chromosomes in these forms into a single *chromosome* group. The linkage of chromosomes, however, can be the cause of extensive genetical

linkage only in case the chromosomes making up the circle are so placed that paternal and maternal chromosomes alternate with one another. If this be true, then, with adjacent chromosomes going to opposite poles, it must follow that all the paternal chromosomes in the circle will go to the same pole in all cells which behave normally, while the maternal chromosomes go to the opposite pole, and but two types of gamete will be formed, as far as the circle is concerned, one of which is strictly paternal in origin and the other maternal. Such a procedure would satisfy the situation as it is known to exist in the majority of wild species, the so-called complex-heterozygotes. It is consequently unnecessary to assume that genes must lie within a single chromosome in order to be linked. Genes lying in all the paternal (or maternal) chromosomes of the circle are linked, so long as the circle remains intact, as effectively as if they were all in the same chromosome.

It should be added that this point of view does not disaffirm the presence of linkage of the ordinary sort, or the possible occurrence of crossing over between genes residing in the same chromosome. It is no doubt true in Oenothera, as in other organisms, that genes which lie in the same chromosome are by necessity linked, and the only way by which they can be separated from one another, so far as we know, is through crossing over. This theory merely adds, therefore, to the first and ordinary kind of linkage, another more extensive, based upon chromosome cohesion.

The second theory is that developed by Professor Shull, who in opposition to the assumption that the chromosomes have a definitely fixed order within the circle, takes the position that "the orientation of the members of a pair is a mere matter of chance, so that in following a series of spiremes from left to right, any chromosome of maternal origin will lie to the left of its paternal homolog in 50 per cent. of the cases, on the average, and to the right in the remaining 50 per cent." (1928). With adjacent chromosomes going as a rule to opposite poles,

the various sets of homologous chromosomes would therefore assort independently, giving rise to the same random mixture of maternal and paternal chromosomes in the daughter nuclei that is to be expected in cases where the homologs are paired. Linkage of the genes in Oenothera has consequently nothing to do with the stringing together of non-homologous chromosomes in meiosis. It is rather to be interpreted in the same way in which it has been interpreted in Drosophila, that is, genes are linked only when they lie in the same chromosome, and become separated from one another only through crossing over. The fact that one of the three linkage groups so far distinguished by this author is very large, quite dominating the others, must therefore be due to the fact that most of the genes affecting visible differences between the different species reside within a single chromosome pair. The rest of the chromosomes are relatively, and in some cases perhaps entirely, free from such genes. Thus we have the conventional explanation of linkage, and the peculiar end-to-end union of chromosomes in the Oenotheras has no genetical significance whatever.

Now, if linked genes in Oenothera are always found within a single chromosome, as the latter theory requires, then linkage relations should in no wise be affected by the type of chromosome configuration that happens to be present in a given instance, since the integrity of the individual chromosomes would not be affected by their arrangement in space in the nucleus. In other words, a group of genes lying within a given haploid chromosome in a certain species should remain linked in all the hybrids of this species, crossing over excepted, no matter what the chromosome configuration, since they will still reside in the same chromosome. The situation is quite different, however, in case the first theory is correct, for if certain genes residing in non-homologous chromosomes are linked merely because the chromosomes in which they lie are always sent to the same pole in the reduction division, then we should expect a linkage based upon such

a situation as this to be maintained only so long as the chromosomes in question remained a part of the same chromosome circle, and the mechanism of distribution was unaltered. But if, as the result of a cross, these chromosomes should come to lie in a hybrid having small circles, and were consequently no longer associated within a single chromosome group, but were separated in diakinesis, so that they were free to assort independently, then we should expect to find a breaking up of this linkage relation in such a hybrid. Hybrids which, like the wild permanently heterozygous species, have circles of twelve or fourteen chromosomes, should resemble these species in passing their complexes on intact, and with little or no indication of independent assortment; whereas on the other hand, hybrids with small circles, and with a considerable number of paired chromosomes, should, on the basis of this theory, show at least some indication of disintegration of the parental complexes and the independent assortment of genes which in the parents had been linked. A method of testing the alternative theories is thus suggested, by comparing the breeding behavior of a series of interspecific hybrids, some of which have large circles and others smaller circles with several pairs of chromosomes. One such experiment has already been reported by Oehlkers (1926), with results favoring the chromosome cohesion theory. It was in order to test the matter further that we have grown and studied a large collection of hybrids.

As we turn, therefore, to a summary of the data bearing upon the breeding behavior of these hybrids, let us have before us two questions: (1) Are there hybrids in which independent assortment actually takes place between genes that were linked in the parents? (2) Is there any correlation between the maintenance or the disintegration of the complexes on the one hand, and chromosome arrangement in diakinesis on the other? If the experimental evidence necessitates an affirmative answer to these questions, confirmation is thereby given to the

theory that chromosome cohesion is at the basis of the extensive linkage resulting in the formation of large complexes in Oenothera.

A brief summary of the genetical behavior of eighteen of the twenty hybrid combinations under consideration (the behavior of the remaining two has yet to be determined) is presented in Table II, in which the various combinations have been arranged in the order of the chromosome configuration. Beginning at the top of the list, we find that nine of the first eleven combinations are similar in chromosome configuration to the permanently heterozygous species that have thus far been studied, having either a circle of fourteen, a circle of twelve and one pair, or a circle of eight plus a circle of six. The remaining two have a circle of ten and a circle of four. Now it has been shown especially by Renner (1925) that one of the most striking peculiarities of permanently heterozygous species is the tenacity with which their gene complexes hang together. The exchange of factors from one complex to the opposing one is a very rare occurrence in spontaneous species. It is most interesting then to discover that the hybrids which in chromosome behavior are identical with these species show the same genetical peculiarity also.

Taking up first those with a circle of fourteen chromosomes, we find no evidence in any that an exchange of factors has occurred between opposing complexes in the F_1, except in the one case of flower size. This is a factor which, according to Renner (1925, p. 154) is probably exchanged between most of the complexes indifferently, and is not coupled with any factor so far as known. From our experience it seems to split as readily in the F_2 of hybrids which have a circle of fourteen as of those with several independent pairs of chromosomes. Aside from this one factor, however, no splitting is to be found in the F_2 of any of the three hybrids listed in the table as having a circle of fourteen, and gene complexes received by these hybrids from father and mother are passed on intact in inheritance, so far as visible characters are concerned.

TABLE II

COMPARISON BETWEEN CHROMOSOME CONFIGURATION AND GENETICAL BAHAVIOR OF CERTAIN F_1 HYBRIDS OF OENOTHERA

No. of unpaired chromosomes	Chromosome configuration	F_1 Hybrid	Extent of factor exchange between complexes of F_1
14	⊙ of 14	truncans. ᵇHookeri (*grandifl.* × *Hook.*)	no exchange (exc. flower size)
		acuens. gaudens (*grandifl.* × *Lamarck.*)	no exchange (exc. flower size)
		albicans. velans (*suaveolens sulf.* × *Lamarck.*)	no exchange (exc. flower size)
	⊙ 10, ⊙ 4	truncans. velans	no exchange (exc. flower size)
		truncans. gaudens & recip. (*grandifl.* × *Lamarck.* & recip.)	exchange of factors for flower size and crippling of main stem
	⊙ 8, ⊙ 6	albicans. gaudens (*suaveolens sulf.* × *Lamarck.*)	probably no exchange (exc. possibly flower size)
12	⊙ 12, 1 pair	deprimens. flavens (*strig.* × *suaveol. sulf.*)	no factor exchange
		gaudens. flavens (*Lamarck.* × *suaveol. sulf.* & recip.)	occasional transfer of flavens lethal, and exch. in fl. size
		albicans. stringens (*suaveol. sulf.* × *strig.*)	no exchange (except pollen sterility factor)
		albicans. elongans (*suaveol.* × *Cockerelli*)	no exchange (according to de Vries)
		albicans. elongans (*biennis sulf.* Hannover × *Cockerelli*)	no exchange (except flower size, to which 1 + genes linked)
		curtans. flavens (*Cock.* × *suaveol.*)	no exchange
10	⊙ 10, 2 pairs	deprimens. velans (*strig.* × *Lamarck. cruc.*)	occasional split in leaf breadth
	⊙ 6, ⊙ 4, 2 pairs	acuens. velans (*grandifl.* × *Lamarck.* & recip.)	considerable factor exchange
		velans. stringens (*Lamarckiana cruc.* × *strig.*)	considerable factor exchange
8	⊙ 8, 3 pairs	flavens. elongans (*suaveol.* × *Cock.*)	occasional exchange of 4 factors
	⊙ 4, ⊙ 4, 3 pairs	acuens. ᵇHookeri & recip. (*grandifl.* × *Hook.* & recip.)	at least 4 independent factors
		velans. flavens (*Lamarck.* × *suaveol. sulf.*)	at least 3 independent factors
4	⊙ 4, 5 pairs	flavens. stringens (*suaveol. sulf.* × *strig.*)	at least 4 independent factors

When we come to hybrids having a circle of ten and a circle of four, or a circle of eight and a circle of six, we might expect dihybrid ratios in the F_2, inasmuch as two apparently independent chromosome groups are present. In the case (*grandiflora* x *Lamarckiana*) *truncans* . *velans*, no split was found, except in flower size. However, the selfed progeny of (*grandiflora* x *Lamarckiana*) *truncans* . *gaudens*, besides showing the split in flower size, included a number of plants in which the main stem was crippled, a variation which might also be interpreted as due to an independent gene. The third combination (*suaveolens sulfurea* x *Lamarckiana*) *albicans* . *gaudens*, has not been studied genetically by us, nor is it mentioned in detail by Renner. The latter states, however (1925, p. 124), that factor exchange, except in flower size, has almost never been observed to occur in any complex-combination involving *albicans*. The same author has furthermore shown that the complexes *gaudens* and *rubens* are practically identical. The combination *albicans* . *rubens* forms the species *biennis*, which breeds true; one would, therefore, expect the hybrid combination *albicans* . *gaudens* to behave in the same way genetically. The fact that both combinations have the same chromosome configuration makes this doubly certain. In view of all this we may assume with reasonable safety that no factor exchange occurs in this hybrid, except possibly in the case of flower size.

When we turn to hybrids having a circle of twelve chromosomes and one pair, we find that factor exchange is still very much restricted. Of those on our list, two show no splitting in the F_2, even in flower size, breeding absolutely true for all known external characters. In a third, the *flavens* lethal is occasionally exchanged, giving rise to *lutescens* forms (Renner, 1925, p. 32). The same being true of the species *suaveolens*, however, this constitutes no new departure. In a fourth hybrid, a single factor, that for pollen sterility, is apparently quite independent of the rest of the complex (Oehlkers, 1926); and

in yet another, there appears to be in addition to the main linkage group another much smaller one (Oehlkers, 1921). In none of these forms do we have more than two clearly independent groups of genes, of which one is small; and in some of them no evidence has been found of the presence of more than a single group.

All the hybrids so far considered have chromosome configurations comparable to those found in the permanently heterozygous species, and it does not appear that the amount of factor exchange taking place in these forms is any greater than that occurring in the species themselves.

As we pass to hybrids with circles of a lower order, however, we find growing evidence of splitting—of independent assortment on the part of factors that in the parent species are linked together. In forms with four chromosome groups, such as (*grandiflora* × *Lamarckiana*) *acuens. velans,* a considerable amount of factor exchange has been found, and the forms at the bottom of the list, with five or six chromosome groups, show strong splitting in the F_2, with as many as four independent factors already recognized in some cases. For instance, in (*grandiflora* × *Hookeri*) *acuens.* [b]*Hookeri,* the factors for flecked leaves, flower size, height and leaf shape are inherited independently of one another. The details of the splitting in the various cases will be published later. We may state at this time, however, that the ratios obtained so far do not as a rule conform closely to the Mendelian scheme. Strictly Mendelian ratios are very difficult to obtain in Oenothera, as Renner has clearly shown. There are no doubt complicating factors present in the genus which disturb the numerical relations. To what extent the splitting in these forms can be shown ultimately to be true Mendelian splitting, modified by certain factors, it is impossible at present to state. We may say, however, that our results strongly suggest the complete genetical independence of at least three or four

factors in those hybrids which have been found to have small circles.

In view of the large amount of data which has been accumulated in this work, the following conclusions, which are submitted subject to further experimentation, may be stated:

1. Hybrids which resemble the wild permanently heterozygous species in chromosome configuration, in that they have large circles during diakinesis, resemble these species also in showing little or no splitting in their progeny. Their gene complexes are passed on intact as far as can be seen from external characters, without any appreciable degree of factor exchange having taken place between opposing paternal and maternal complexes. But with increase in the number of free chromosome pairs comes an increase in the amount of splitting in the progeny of the hybrid, and hybrids which possess small circles, with several independent pairs of chromosomes, produce progenies in which a considerable amount of variation occurs. The parental gene complexes become broken up, and independent assortment appears to take place between factors that in the parents were linked hard and fast.

2. The existence of a definite correlation between the presence or absence of extensive chromosome cohesion, on the one hand, and the presence or absence, respectively, of extensive genetical linkage on the other, constitutes strong evidence in favor of the theory that chromosome cohesion is at the basis of extensive linkage and the formation of but two kinds of gene complex in each of the species of Oenothera. It is not likely that such a correlation can be explained on the assumption that linkage is confined entirely to genes residing in a single chromosome pair.

3. It follows that genes for visible characters are more or less evenly scattered throughout the various chromosomes, and are not confined for the most part to a single chromosome pair.

LITERATURE CITED

Cleland, Ralph E.

1922. "The Reduction Divisions in the Pollen Mother Cells of *Oenothera franciscana*," *Amer. Jour. Bot.*, 9: 391–413.

1923. "Chromosome Arrangements During Meiosis in Certain Oenotheras," AMER. NAT., 57: 562–566.

1924a. "Meiosis in the Pollen Mother Cells of *Oenothera franciscana sulfurea*," *Bot. Gaz.*, 77: 149–170.

1924b. "Chromosome Behavior During Meiosis in the Pollen Mother Cells of Certain Oenotheras," *Anat. Rec.*, 29: p. 132.

1928. "The Genetics of Oenothera in Relation to Chromosome Behavior, with Special Reference to Certain Hybrids," *Verhandl. d. V. internat. Kongr. f. Vererbungswissensch.*, Berlin, 1927: pp. 554–567

Gates, R. Ruggles.

1928. "The Relations of Cytology to Genetics in Oenothera," *Verhandl. d. V. internat. Kongr. f. Vererbungswissensch.*, Berlin, 1927: pp. 749–758.

Håkansson, A.

1926. "Ueber das Verhalten der Chromosomen bei der heterotypischen Teilung schwedischer *Oenothera Lamarckiana* und einiger ihrer Mutanten und Bastarde," *Hereditas*, 8: 255–304.

Oehlkers, Fr.

1921. "Vererbungsversuche an Oenotheren I. *Oenothera Cockerelli* Bartlett und ihre Kreuzungen," *Zeitschr. f. indukt. Abst. u. Vererb.*, 26: 1–31.

1923. "Vererbungsversuche an Oenotheren II," *Ibid.*, 31: 201–260.

1926. "Erblichkeit und Zytologie einiger Kreuzungen mit *Oenothera strigosa*," *Jahrb. f. wiss. Bot.*, 65: 401–446.

Renner, O.

1925. "Untersuchungen über die faktorielle Konstitution einiger Komplexheterozygotischer Oenotheren," Bibliotheca Genetica, Vol. 9.

Sheffield, F. M. L.

1927. "Cytological Studies of Certain Meiotic Stages in Oenothera," *Ann. Bot.*, 41: 779–816.

Shull, Geo. H.

1928. "Oenothera Cytology in Relation to Genetics," AMER. NAT., 62: 97–114.

DURATION OF THE EFFECTS OF X-RAYS ON MALE GERM CELLS IN *DROSOPHILA MELANOGASTER*[1]

DR. FRANK BLAIR HANSON AND FLORENCE HEYS

WASHINGTON UNIVERSITY

DURING the past two years it has been abundantly established by Muller,[2] Stadler,[3] Weinstein,[4] Whiting,[5] Hanson and Heys[6] and others that X-rays and radium produce gene mutations in the several organisms studied. In Drosophila both visible and lethal mutations result from such treatment.

In Muller's earliest experiments the flies which hatched from eggs laid seven to twelve days after raying of the sperm which fertilized them showed the same percentage of mutations as those hatching from eggs fertilized by sperm during the first six days following treatment.

This raises the interesting problem of the duration of the effects of irradiation upon mature sperm and also whether there is a differential effect of the same dose upon the various stages of spermatogenesis.

The first of these questions has been answered by some experiments of B. B. Harris, of the University of Texas. Dr. Harris has kindly allowed me to refer to his results in advance of their publication. He found that when sperm are aged in the male and no copulation allowed

[1] The expenses of this investigation were supported in part by the committee on the effects of radiation upon living organisms of the National Research Council.

[2] H. J. Muller, "Artificial Transmutation of the Gene," *Science*, 66: 84–87, 1927.

[3] L. J. Stadler, "Mutations in Barley Induced by X-rays and Radium," *Science*, 68: 186–187, 1928.

[4] A. Weinstein, "The Production of Mutations and Rearrangements of Genes by X-rays," *Science*, 67: 376–377, 1928.

[5] P. W. Whiting, "The Production of Mutations by X-rays in Habrobracon," *Science*, 68: 58, 1928.

[6] Frank Blair Hanson and Florence Heys, "The Effects of Radium in Producing Lethal Mutations in *Drosophila melanogaster*," *Science*, 68: 115–116, 1928.

except during certain specified periods no significant decrease in the mutation rate occurs even up to twenty days after raying. For instance, males which were allowed to copulate one to ten hours after treatment gave 10 per cent. lethal mutations, while those which were withheld from females for fifteen days after raying and then were mated from the sixteenth to the twentieth days gave a rate of 9 per cent. That these sperms used from sixteen to twenty days after raying were mature when treated is indicated by results given below. It seems valid to conclude that when sperm are aged in the male the effects are permanent at least for twenty days, which is a considerable portion of the total productive period in Drosophila.

Harris and the authors have independently studied the effects of the *same* dosage of X-rays, and the writers also the effects of radium, upon early and late stages in male germ-cell formation.

In these experiments Muller's *ClB* stock of flies which gives an exact percentage of lethal mutations was used. Much more accurate results quantitatively can be secured with lethals than with visibles, where the personal equation enters more largely into the results.

Wild type males were exposed to radium in the first experiment and to X-rays in the second.

Immediately following treatment these males were mated, in pairs, to females having the genes for scute (sc), vermilion (v), forked (f), and bobbed (bb) in one X-chromosome and a gene (C) to prevent crossing over, a lethal gene (l), and the bar gene (B) in the other X-chromosome. The F_1 females were of two classes, bar and not-bar. The bar-eyed females were mated to their brothers, one pair to a tube, to produce the F_2 generation. Due to the presence of the lethal gene in half of the sons in this generation they never hatch. If a new lethal mutation arose as the result of the radium treatment the other half of the sons would inherit it and not appear. Hence no males would hatch in that particular tube. To determine the number of lethal mutations occurring as a

result of the exposure to radiation it was only necessary to count the number of tubes in which there was 100 per cent. females.

The Radium Experiment

Using the above-described breeding methods, males were exposed to 150 mg of radium for nine hours and then mated to virgin ClB females, one pair to a bottle. These pairs remained together for seven days in the same bottles and then were transferred to new food bottles for seven days more. Transfers to new food bottles were made at such seven-day intervals for a period of thirty-five days. The young hatching from eggs laid during the first seven days after raying are known as Brood I, those of the second seven days constituted Brood II, etc. There were, therefore, five broods in all, and the mutation percentage of each brood is given in Table I.

TABLE I

Broods	Age of germ cells in days after treatment with radium	Percentage of lethal mutations
I	1– 7	13.00
II	7–14	14.20
III	14–21	1.01
IV	21–28	4.30
V	28–35	0.00

Table I shows clearly that for fourteen days after irradiation there is no appreciable change in the mutation rate. In the period fourteen to twenty-one days, a sharp drop occurs, the percentage of lethals decreasing from 14.2 in the preceding brood to 1.01 in this third brood. Broods IV and V give a mutation rate of 4.30 and 0.00 respectively.

It appears that this dose of radium has a differential effect upon early and late germ-cell stages. It is suggested, in the present state of our knowledge, that the mature sperm present at the time of treatment lasted for approximately fourteen days when the males were con-

tinuously mated. Subsequent to this, fertilization was accomplished by sperm which during treatment were in the earlier stages of spermatogenesis. The radium seems to have had much less effect upon early than upon late male germ cells.

THE X-RAY EXPERIMENT

In the radium experiment the dosage given was a severe one, causing much complete sterility and, hence, reducing the number of F_2 tubes below what was considered desirable for such a test. The experiment with X-rays was carried out in the same manner as the radium experiment with regard to breeding methods, etc., the only difference being that X-rays were substituted for radium and a dose approximately one half the strength of the radium dosage was used. This experiment was carried through four broods only as the critical point seemed to be between broods II and III. Table II gives the results of the X-ray experiment.

TABLE II

Broods	Age of germ cells in days after treatment with X-rays	No. F_1–F_2 bottles	Percentage of lethal mutations
I	1– 7	504	6.5
II	7–14	605	6.4
III	14–21	614	2.9
IV	21–28	470	2.1
	Controls	1308	0.000764

In experiment II, it is clear that there is a distinct drop after the second week, as in experiment I. The weaker dose of X-rays employed is reflected in the percentage of lethals for Broods I and II as compared with the corresponding broods for the radium experiment.

In a similar experiment Harris got confirmatory results and thinks that the mutation rate is from five to ten times greater in mature germ cells than in immature germ cells. Our radium experiment gives a larger dif-

ference than this, while the X-ray experiment gives a smaller difference. Since dosages of X-rays and radium can not be directly compared, and Harris used a four-day interval instead of the seven-day interval used here, the discrepancies are not serious. In fact, both sets of experiments are clear-cut in showing a differential effect of the same dose of radiation on immature and mature germ cells. However, the effect was not wholly restricted to mature germ cells, and apparently some mutations came through from all stages of spermatogenesis. It may be assumed that sperm which fertilized eggs (Table I) twenty-eight to thirty-five days after raying were in a very early stage at time of treatment, probably spermatogonia.

Harris has kindly permitted the publication of his table for comparison with our Tables I and II.

HARRIS'S DATA ON AGE EFFECTS

Age of germ cells in days after raying	No. F_1–F_2 vials	Percentage lethals
1– 4	1206	8.6
4– 8	886	9.7
8–12	875	7.3
12–16	960	1.7
16–20	823	0.6
20–24	740	0.8
Controls	617	0.2

Harris's X-ray dosage was somewhat heavier than ours as indicated in the percentage column of his table. Using a four-day interval the sharp drop comes in his data during the period twelve to sixteen days, while in our two experiments it was in the period fourteen to twenty-one days. Our Brood III (The X-ray experiment) indicates that there was still some admixture of sperm that were mature at the time of treatment. A relatively cool temperature would tend to give the same results by making each week count as equivalent to a smaller number of days at a higher temperature. Our

cultures were kept at room temperature and this dropped somewhat over night.

CONCLUSIONS

1. When irradiated sperm are aged in the male and no copulation allowed except during certain specified periods no decrease in the mutation rate occurs.

2. When irradiated males are continuously mated those sperm mature at time of treatment function for approximately two weeks, as indicated by the constant mutation rate, after which there is a sudden and sharp drop in the rate, presumably due to fertilization being accomplished by sperm that were immature at time of treatment.

3. There seems to be a differential effect of the rays upon immature and mature male germ cells.

THE TUBE FEET OF STARFISHES AS AUTONOMOUS ORGANS

VIRGINIA L. PAINE

ZOOLOGICAL LABORATORY, RADCLIFFE COLLEGE

I. INTRODUCTION

IT is well known that the tube feet of starfishes, even after they have been cut off from the animal, are capable of independent motion. This independence is indicative of a certain amount of muscular and perhaps nervous autonomy. Similar autonomous conditions have already been recognized in the various organs of a wide range of animals as, for instance, in the heart of the higher metazoans, the small intestine of vertebrates (Mall, 1896), the foot of gastropods (Jordan, 1901, 1904), the tentacles of sea anemones (Parker, 1917), the palps of lamellibranchs (Matthews, 1928), and in many other such parts. In the course of my study on the power of adhesion of the tube feet in the starfish (Paine, 1926) I was impressed with the autonomy of these organs and I therefore undertook a study of them with the intention of ascertaining their degree of autonomy and the finer structure on which it depended. I used for this investigation the common starfish of the New England coast, *Asterias vulgaris* Verrill, which was obtained from the rock pools at Nahant, Massachusetts. Most of the work was done in the zoological laboratory of Radcliffe College, under the direction of Dr. G. H. Parker, but some of it was carried out during the summer at my residence at Nahant.

II. THE AUTONOMY OF THE TUBE FEET

The autonomy in starfish feet is to be observed in single feet which have been cut off from the animal. Such feet lack ampullae and are open at the proximal end. They continue to live and are capable of movement for several days provided they are placed in bowls of sea

water which is kept cool and either well aerated or frequently renewed. However, the most favorable time for observing reactions is the first day after the feet have been cut off, though occasionally response to definite stimuli is as strong as ever on the second day. After this time, the only reaction is an extremely slow contraction and elongation—one foot after four days responded in this way—but for the most part the feet remain contracted until disintegration begins. Immediately after they are cut off from the starfish, the feet contract quickly and remain contracted for several minutes. After this lapse of time they generally elongate very slowly, by an almost imperceptible movement. A little later, though they are given no definite stimulation, they begin to move in various ways: contracting slowly, bending, curving or elongating still further. Occasionally the sucker, at the distal end, curves over toward the bottom of the bowl and the surface of the sucking disk wrinkles slightly, as it does when about to take hold of an object; but I have never seen a foot which had been cut off actually attach itself to the bottom. Any of these movements are to be observed from time to time during the first day, and sometimes on the second day. The liveliness of the single severed feet and the length of time during which they react are apparently determined by the temperature of the water, the amount of oxygen present, the season of the year and other like factors. It seemed impossible to know when conditions were especially favorable or adverse for experimentation. Feet taken from animals previously kept out of doors near the laboratory for several days, or even a week, are often as active as those from animals that had been in their native pools up to the time of experiment.

Isolated feet contract when they are touched by a glass rod or a probe—the rate of contraction, although generally low, is higher than when they contract without being touched. Rarely, when a glass rod is brought into contact very gently with the sucking surface and held

there, the disk adheres to the rod and clings with enough force to allow the foot to be lifted from the water. After a second or two, however, the foot loses its hold and drops back. A slight wrinkling of the sucking disk suggests that suction has been used, yet it is possible that the foot adheres solely because of the mucus exuding from it. Probably the large and numerous mucous glands of the sucker are stimulated by touch, as at such times there are little strings of mucus clinging to the sucker, visible when powdered carmine is sifted on the preparation. Twice only, excised feet stuck to a small piece of glass used for registering the pull of the foot, and readings were obtained of five and eight grams. A curious feature about these feet was that one of them had been in a solution of magnesium sulphate and the other in a solution of potassium iodide for some time before they were tested, and that this treatment seemed to make them cling more tightly than is usual. This peculiarity will be considered in connection with certain observations to be described later. A weak electric current appeared also to induce feet to cling to a glass plate. The reaction of excised feet to various chemical stimuli, such as weak acetic acid, weak hydrochloric acid, lemon-juice, weak alcohol, crushed nightshade berries, sugar, etc., was tried, and it was seen that all cause a fairly sudden contraction—very much quicker than that caused by touching the feet with a rod. A very slight rise in temperature made the feet elongate slowly and remain in this condition, while ice put in the sea water brought about a shortening. Chlorotone acts as an anesthetic, causing relaxation and consequent elongation. The action of magnesium sulphate on isolated feet is puzzling. Up to a certain point of concentration it increases the activity of the foot; the pulling power of the sucker particularly seems to be temporarily greater; when more magnesium sulphate is added the foot contracts, but after several minutes it elongates and is apparently anesthetized. It is also interesting to find that when a whole starfish is

put into a solution of magnesium sulphate the feet are apparently the last part of the animal to become anesthetized, and when the animal is put back into the sea water, the feet are the first part to loose this condition.

Novocaine seems to have no anesthetizing effect on isolated feet. When used in about .01 per cent. solution on a whole animal it prevents coordination; for instance, animals in it become very still except for the absolutely free movement of the feet, and are unable to right themselves when turned on their backs. Animals in novocaine stop crawling and the rays move very little and finally not at all. The feet, however, are very active and either wave about or occasionally cling to the glass. They are very responsive to stimuli. In fact, it seems to me that they are even more responsive than normally —this may perhaps be accounted for, if true, by supposing that when centers of general coordination are anesthetized the nerve centers of the feet, being independent, are freed. A comparable condition was noticed in the foot and wings of Aplysia by Jordan (1901). Carbon dioxide gave interesting results. When a whole animal was put into carbon dioxide water it responded by crawling quickly round and round the bowl and bending up the ends of its rays at sharp angles. Single excised feet bent quickly when the carbon dioxide was added, but they did not contract, and responded naturally on being touched. After one minute all the feet contracted and, as long as fifteen minutes afterwards, while still in the carbon dioxide solution, responded in the usual way to stimuli.

Juice of a crushed mussel dropped in the water near or on isolated feet brought no response, nor did small pieces of mussel or whole mussels. At the same time, the animals from which the feet were taken were stimulated by the same kind of food materials, and the feet took hold of the solid pieces and passed them to the mouth. Feet on the animal responded to mussel juice by waving around and then bending toward the mouth.

Beef juice also stimulated whole animals to a high degree, but had no effect on isolated feet.

When a starfish is clinging to the side of a dish and is pulled off, it frequently leaves dozens, even hundreds, of its feet behind, sticking to the glass. Observation shows that generally such feet cling merely for a few seconds or minutes, but occasionally I have seen them remain stuck to the glass for almost an hour. Once, on a cool morning in Nahant, I pulled off a medium-sized starfish which was clinging to the side of a bowl. A complete pattern of its rays was left, made by the feet that broke off and still clung to the glass. In less than fifteen minutes about three quarters of the feet had let go and floated off; by an hour, all but thirty feet were gone. Five fairly long feet and a dozen sucking disks, broken off short, remained clinging for nearly two hours in all. Other measurements of time to determine how long feet would cling were taken on the same morning and generally the time was longer than the average of any other day for which I made note of the time.

In most cases, however, it looks as though suction ceases almost as soon as the feet are separated from the animal, but this does not always happen as is shown in the observation just described. While the feet are still attached to the dish, they are not dislodged by dropping weak hydrochloric acid or alcohol on them, but if touched they fall off. When I added magnesium sulphate to the water in which were isolated feet already clinging to the dish, they continued to cling for as long as fifteen minutes.

If the sucking disk is cut off from a foot, the disk is able to move by itself, wrinkling and bending up its edges, and responding in these ways to mechanical stimulus. A small isolated piece of foot taken from any part of the organ contracts and curls when touched.

Matthews (1928) found that isolated labial palps of Anodonta, in which he demonstrated the presence of a nerve-net, responded more quickly and more strongly to

stimuli than they did when still on the animal, and Jordan (1901) in his work on Aplysia found that certain muscular parts separated from the central nervous system were much more active and had a higher tone than they had when still normally connected. My experience with excised feet of starfishes was contrary to this.

Probably this lessened efficiency was due to the fact that in such specimens connections with the water-vascular system, which plays an important part in locomotion, were broken. This seems more likely, as I made some observations of cut-off feet to which the ampullae were still attached. These feet were certainly fully as lively and efficient as feet still on the animal, and were remarkably active—almost never remaining still. Such feet were on the whole, however, less suitable for studying autonomy than feet cut off below the ampullae, because of the impossibility of removing completely the ray skeleton.

Under almost all conditions the movements and reactions of excised starfish feet and even pieces of such feet were similar to those of feet still attached to the animal, though the responses of the isolated feet were always weaker and slower than those of attached feet. This falling off in efficiency may be associated with the water-vascular system, whose water pressure, even though slight, is undoubtedly of significance in the normal movement of the feet. This also is suggested by the fact that when an animal is in novocaine and the centers of coordination anesthetized the feet respond as strongly, or seemingly more strongly, to stimuli and are more efficient apparently than those of an animal in plain sea water. The same thing is apparently true in the first stages of anesthetization with magnesium sulphate. It must be kept in mind that isolated feet do not respond to mussel or beef juice and bits of mussel as attached feet do. Isolated feet react, however, to strong chemical stimuli.

III. Structure of the Tube Feet in Relation to their Activities

The general histological structure of the tube foot of the starfish has long been known. It can be conveniently demonstrated in sections from material fixed in Flemming's chrom-acetic-osmic mixture and stained in iron hemotoxylin. Fixation by so simple a means as hot water also gives on the whole satisfactory preparations. Maceration in 20 per cent. nitric acid followed by staining with iron hemotoxylin has yielded interesting results. For nervous elements, as will be described subsequently, methylene blue used as an *intra vitam* stain proved important.

The foot, as shown in longitudinal section (Fig. 1), is covered with a moderately thick epidermis (EP) con-

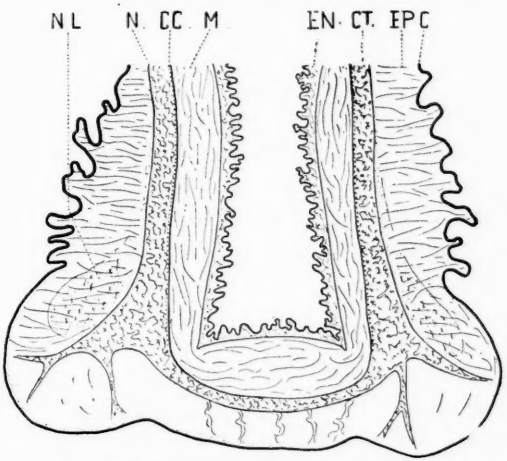

Fig. 1. Diagram of a longitudinal section of the tube foot of a star-fish; C, cuticula; CC, circular connective tissue; CT, loose connective tissue layer; EN, endothelium; EP, epidermis; M, longitudinal muscle; N, nervous layer that enlarges considerably; NL, near the free end of the foot.

taining numerous large mucous cells, many long fibrous cells, probably of a sustentative nature, among which are what appear to be sense cells. The epidermis is covered externally with a thin, transparent cuticula (C). On the

inner face of the epidermis is a layer of very fine fibers, probably nervous in nature (N). Toward the distal end of the foot this enlarges and forms a ring-like structure (NL), the so-called "nerve ring," as described in *Asterias rubens* by Hamann (1885) and by Chadwick (1923); both believed this structure to be continuous with the radial nervous system of the starfish arm.

Next to the epidermis, with its basal layer of nervous material, lies a loose and rather porous layer of connective tissue (CT), which becomes relatively compact, very strong and fibrous in the region of the sucker. Inside this supporting layer is a second layer of connective tissue (CC), thin, compact, and with fibers whose course extends in a circular direction around the foot. Inside these connective-tissue layers is a sheet of longitudinal muscle fibers (M), which at the distal end of the foot turns at right angles and spreads over the sucking disk, thickening as it approaches the center. As this is the only muscle I was able to find in the foot, I believe it to be the essential element in the bending of the foot, in suction and in other movements. The fibers of this muscle, stained with iron hemotoxylin, exhibit, both in sections and in maceration material, cross striations that are so regular as to suggest an actual structure. Inside this layer of muscle is a folded endothelium (EN) which lines that portion of the water-vascular system that extends into the tube foot.

Since no circular muscles were identified in the cylindrical wall of the foot, it is difficult to understand by what means the foot elongates. Special histological technique to determine the presence of elastic tissue revealed nothing; but as the technique was devised for vertebrate tissue, negative results meant little. Feet long anesthetized and relaxed in chlorotone were artificially stretched lengthwise; when they were released they contracted to their original form. As the anesthetic prevented ordinary muscular movement, what contraction there was must have been due to the elasticity of the tissue.

Under natural conditions elongation of the feet still on the animal may depend solely on hydrostatic pressure; but such can not be the case in an isolated foot whose connection with the water-vascular system has been destroyed. I did not find any calcareous material or other hard skeletal parts in the foot, but a great deal of support must be given by the thick and compact connective tissue at the base of the foot and by the sustentative fibers in the epidermis.

The cavity of the foot is continuous with that of the water-vascular system, and each foot is connected with an ampulla by a narrow, very thin-walled neck in which I have failed to see any valve. While the foot is still attached to the animal the ampulla and foot generally work together. When the ampulla contracts some of the water is forced into the foot which causes it to elongate, and when the foot contracts the water is returned to the ampulla. Though it has been shown that a foot without an ampulla is capable of almost all normal movements, the presence of an ampulla greatly increases the strength and speed of such activities and the length of time over which they can be carried out. Single, living feet, connected with their ampullae, were dissected out and isolated. Except for the very small opening that leads into the radial canal, and where leakage is apparently prevented by the presence of a small valve situated between the foot and the canal, the foot and ampulla together make a closed system and a complete unit in a mechanical sense. It is probably because of this mechanical efficiency rather than because any new nervous element is added that feet with ampullae are so much more active than those without. However, the ampullae of these isolated feet very seldom actually work with them. Two or three times I have seen an ampulla contract when a foot expands or an ampulla expand when a foot contracts, but on the whole the ampulla remained without motion. It seems possible to me that if the ampulla could be tied off

just above the foot, and cut off both from it and the animal, more autonomy might be shown.

The movements of the isolated feet suggest the presence of nervous elements in the feet themselves. From the physiological resemblance to other autonomous organs, such as the tentacles of the sea-anemone, the labial palps of clams, etc., it seems likely that such nervous elements would take the form of a nerve-net. When a foot is cut so that the incisions alternate first from one side and then from the other, thus interrupting the continuity of possibly direct nerves, the foot still continues to show a spread of contraction. This condition confirms the idea of a nerve-net.

Ordinary nerve-stains for fixed tissue revealed nothing of importance, hence *intra vitam* staining with methylene blue was used. The method followed was, for the most part, the one described by Cole (1925) and adapted in concentration and time to starfish tissue. Ehrlich's BX methylene blue was generally the most successful, but methylene blue from the National Analine and Chemical Company gave very good results. The dye was dissolved in physiological salt solution or in sea-water. Single feet were cut off and put in a watch-glass containing a saturated solution of methylene blue in either sea-water or physiological salt solution. After somewhat over an hour the feet were taken out singly, cut longitudinally and gently passed flat under a cover-glass. The fresh preparation was then examined under the microscope and such nerve cells as were to be clearly made out were observed and drawn.

This method was not uniformly reliable, and its success apparently depended on many conditions that were not under complete control. Feet cut from very fresh and active starfish are less apt to stain than those taken from animals in a less vigorous state. The dye seems to work much better in warm weather. There were weeks, and even months, when I could not get a single successful result; yet during August and September at Nahant and

in June at Cambridge I had satisfactory preparations daily. Though examination of the preparations before fixation was most important, I wished to retain the staining and make it permanent. I followed Cole (1925) in fixing the tissue with ammonium molybdate in Ringer's solution. Whole mounts of feet cut longitudinally and pressed flat, as well as serial sections, were made.

When these preparations were examined, typical sense cells were found, especially in or near the sucker, in the outer part of the epidermis (Fig. 2). These cells lose

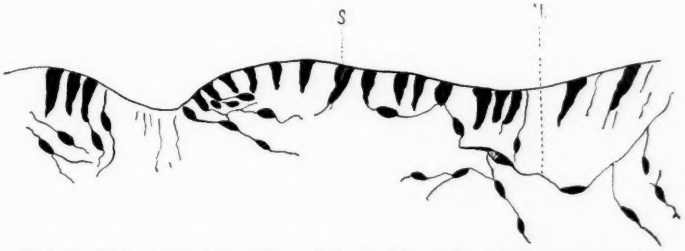

FIG. 2. Epithelium from side of tube foot immediately above sucker showing numerous sense cells (S) whose fibrous ends connect with the subepithelial nerve-net (N). Stained with methylene blue *intra vitam* and cut in paraffin.

much of their detail in fixation, but as the staining of fresh material lasts only a few minutes, and it is also impossible to locate anything with any exactness in it as only one tissue is stained at a time, I have made the accompanying drawing from a section. Large sense cells at the outer edge of the epidermis appear to be connected by longer or shorter processes with other nervous cells lying deeper in the epidermal layer. These deeper cells certainly seem to unite and form a net (N). The nuclei of such cells are scarcely larger than those of connective tissue cells, but differ from them in shape, and the whole cell, with its small amount of cytoplasm around the nucleus and its long, fine processes, is quite distinctive. When I compare these cells and their arrangement with the description of similar cells found in other animals, it

seems likely that they are parts of a nerve-net such as has been found in other autonomous organs. The net-like appearance was not as perfect and general in distribution, however, as I have seen it in drawings taken from organs of other animals, but it was sufficiently clear to give the decided appearance of a true net.

Similar nerve cells are also found in the ampullae, and I was able to demonstrate them in several preparations.

There is no question that from a physiological standpoint, starfish feet are autonomous organs. They are capable of long and varied movement after they are cut off from the animal and respond naturally to many stimuli for days. An entire starfish may be anesthetized without affecting the feet, which will continue to move freely. This autonomy can not be dependent on muscle alone or on any continuity of direct nerves as was shown by making alternating cuts in an isolated foot. Such response could take place through a nerve-net and probably not in any other way.

Histologically, typical sense cells are found at the outer edge of the epidermis, and these cells connect by processes to other cells lying in the deeper part of the epidermis. These cells, to every appearance, anastomize clearly and form a net. When epidermal sense cells connect with deeper-lying cells forming a net and within this a muscle layer is present, we have all that is needed for the nervous autonomy of a given organ such as the tube foot of a starfish.

SUMMARY

From both the physiological and histological aspects of the tube feet of *Asterias vulgaris,* it is clear that they are autonomous organs. They are capable of varied and long response to stimuli when excised, although the response of a foot without an ampulla is less than when the feet are normally attached. However, this is probably due to destruction or injury of the water-vascular connections. Isolated feet with ampullae still attached

lose none of the efficiency that normally attached feet show. The use of anesthetics that influence the animal as a whole, but leave the feet free, also strengthens the idea of an independent nervous structure in the feet. Isolated feet, cut alternately on one side and on the other so as to destroy any continuity of direct nerves, if stimulated, contract, showing that it is still possible for transmission to take place, and that this must be through some tissue whose continuity is not affected by the cuts, probably a nerve-net. Histological examination confirms this, as sense cells are found at the outer part of the epidermis, and these are connected by processes with other cells, seemingly of nervous nature, lying deeper in the epidermal layer. These latter form a loose net. The appearance of the whole agrees with the descriptions of the nerve-net found in other animals.

LITERATURE CITED

Chadwick, H. C.
1923. "Asterias." Liverpool Mar. Biol. Comm., Memoirs on Typical British Plants and Animals, XXV.

Cole, E. C.
1925. "Anastomizing Cells in the Myenteric Plexus of the Frog." *Jour. Comp. Neur.*, 38: 375–387.

Hamann, O.
1885. "Beiträge zur Histologie der Echinodermen." Heft 2, "Die Asteriden." Jena, 126 S.

Jordan, H.
1901. "Die Physiologie der Locomotion bei *Aplysia limacina*." *Zeit. Biol.*, Bd. 41, S. 196–238.
1904. "Untersuchungen zur Physiologie des Nervensystems bei Pulmonaten." *Arch. ges. Physiol.*, Bd. 106, S. 189–228.

Mall, F.
1896. "A Study of the Intestinal Contraction." *Hopkins Hosp. Rep.*, 1: 39–75.

Matthews, S. A.
1928. "The Palps of Lamellibranchs as Autonomous Organs." *Jour. Exp. Zool.*, 51: 209–258.

Paine, V. L.
1926. "Adhesion of the Tube Feet in Starfishes." *Jour. Exp. Zool.*, 45: 361–366.

Parker, G. H.
1917. "The Movements of the Tentacles in Actinians." *Jour. Exp. Zool.*, 22: 95–110.

STUDIES ON THE LIFE HISTORY OF THE BLUNT-NOSED MINNOW[1]

PROFESSOR HARLEY J. VAN CLEAVE AND HENRY C. MARKUS
UNIVERSITY OF ILLINOIS

WITHIN recent years the life histories of a considerable number of species of marine and fresh-water fishes of direct economic importance have been studied in detail. The discovery of a method of interpreting the age of fishes through evidences provided by the growth rings upon the scales has been one of the potent factors stimulating research in this field. Though the minnows are one of the characteristic families of North American fishes, very little is to be found in the literature regarding their life cycles. There are numerous brief notes on the breeding habits and on egg laying and some observations on the rate of growth during a single season, but specific information on the life cycle as a whole seems lacking. It is the purpose of this paper to present an analysis of data pertaining to the blunt-nosed minnow, a species very widely distributed over eastern United States. At present, ichthyologists are using the name *Hyborhynchus notatus* (Rafinesque) for the blunt-nosed minnow, though in much of the literature this species has been known as *Pimephales notatus.*

MATERIAL

On April 27, 1928, Dr. David H. Thompson, of the Illinois State Natural History Survey, and the senior author of this paper secured an especially rich collection of the blunt-nosed minnow from the East Branch of the Black Slough, a dredged tributary of the Embarrass River. The locality where the collection was made is about eight miles south of Urbana, Illinois. A large roll of discarded wire fencing lay in a pool formed by the widening of the

[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 345.

channel of the stream. This artificial shelter seemed to provide an unusually favorable habitat for the minnows, for 369 individuals of the one species, *Hyborhynchus notatus,* were driven from this cover into a minnow seine. The entire collection was preserved in formalin and seemed to offer exceptional opportunity for an investigation of certain aspects of the life history. Some of the smallest individuals, comparable in size to the smallest of the preserved specimens, escaped through the meshes of the seine. Consequently, statistical data based upon relative numbers of the size groups are invalidated.

The specimens available for measurement ranged in size from 1.3 to 7.9 cm. Dr. Thompson has very kindly provided us with unpublished data on the frequency of occurrence and maximum size of *H. notatus* in this immediate vicinity. In his collections, extending over a period of two years, he has amassed a series of 6,440 specimens of *H. notatus* from Champaign County alone. In this extensive series there is no individual larger than the 7.9-cm specimens taken from the Black Slough tributary. Since Forbes and Richardson give three and one half inches as the maximum length of this species, the largest specimens under consideration do not fall far below the maximum size recorded for the species in Illinois and represent the maximum attained by the species for the immediate locality under consideration. It thus seems evident that the single collection which forms the basis for the present investigation includes unusually favorable material for statistical approach to a life history study.

In the present paper only a few of the many topics in the life cycle have been considered. These comprise the analysis and correlation of size, sex and age and observations on the secondary sexual characters of the male.

Methods and Results

Preliminary to further study, the specimens were sorted into size groups in the hope that such a separation might throw some direct light upon age groups of the

individuals. Though this separation into size classes did yield some interesting information, as shown in Fig. 1, the full significance of the distribution in the frequency curve did not become apparent until a further analysis of the data concerning sex and the evidences of relative age had been made.

In Table I, the irregularity in the increments of mea-

TABLE I

DISTRIBUTION OF 369 SPECIMENS OF BLUNT-NOSED MINNOW BASED UPON SIZE, SEX AND DEVELOPMENT OF THE SECONDARY SEXUAL CHARACTERS OF THE MALES

Group number	Average size of specimens in centimeters	Number of specimens	Number of males	Number of females	Per cent. males	Per cent. females	Number of males showing secondary sexual characters	Number of specimens with immature gonads
1	7.9	3	3		100		3	
2	7.6	5	5		100		5	
3	7.4	38	38		100		38	
4	7	35	35		100		17	
5	6.7	31	31		100			
6	6.2	21	21		100			
7	5.7	22	15	7	68.18	31.82		
8	5.3	47	7	40	14.89	85.11		
9	5	78	1*	77	1.28	98.72		
10	4.8	28		28		100		
11	4.5	19		19		100		
12	4.2	3						3
13	3.8	4						4
14	3.5	11						11
15	3.2	6						6
16	3	9						9
17	2.7	4						4
18	1.3	5						5
Total		369	156	151			63	42

* Head deformed.

surement for the size groups should be explained. The collection seemed most readily divisible into natural groups, so these were chosen for sorting rather than uniform increments of size. One of the largest individuals was selected and measured by means of a pair of dividers. All specimens not deviating from this length

more than 1 mm were separated into a group. Throughout this study the length is considered as the distance from the tip of the snout to the end of the vertebral column at the base of the tail. The entire collection was distributed into eighteen size groups, as shown in Table I. On the basis of this distribution of the total population, a frequency curve was prepared (Fig. 1). An in-

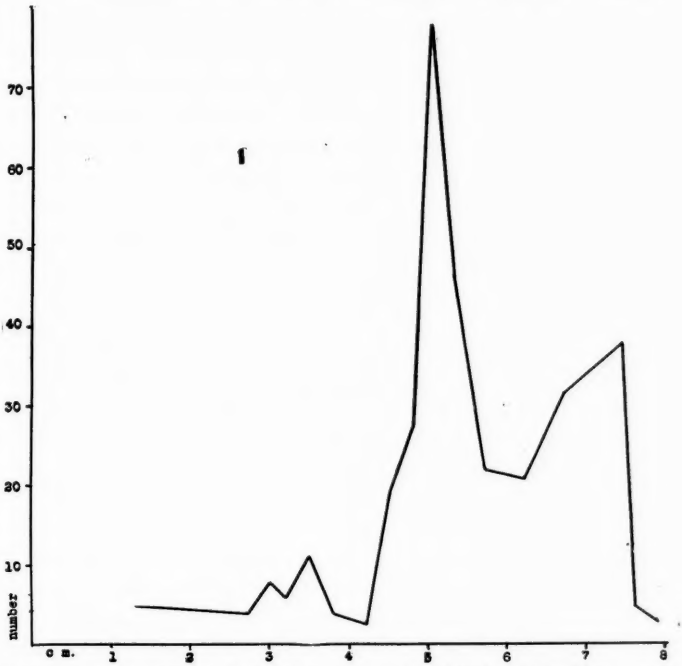

FIG. 1. Graph showing distribution of 369 individuals of the blunt-nosed minnow according to lengths in centimeters.

spection of this graph immediately suggested the possibility that the individuals under 4 cm in length are the young which have passed through a single winter and are nearing the close of their first year. This conclusion was confirmed by further studies involving microscopic examination of the scales as described later in this paper.

The bimodal nature of the graph in the region of the larger specimens became evident at once and suggested the alternatives that either age or sex might be responsible for the establishment of two distinct modes. Through an inspection of Table I, it had already been noted that all the largest individuals bore pearl-organs, or tubercles on the head, which have been generally admitted as distinctive of the male sex. It was consequently decided that the sex of each individual should be determined by a direct examination of the gonads.

Dissections revealed the fact that every individual over 6 cm in length was male. No female exceeded 5.7 cm. The median size of the females with well-developed ovaries was 5 cm; for males with distinguishable testes, 7 cm. Though the ages of these specimens will be discussed in greater detail in a later part of this paper, it should be mentioned here that the largest males are within a few months of three years old, while the largest females are but slightly under two years of age. Even when we compare males and females of the same age, the disparity in size persists, for the males nearing the close of their second year exceeded the length of the oldest females by more than 1 cm.

Among fishermen, it seems to be pretty generally the opinion that in fishes the females are larger than the males. Günther, in his widely quoted "Introduction to the Study of Fishes," gives support to this current belief in the words: "With regard to size, it appears that in all Teleosteous fishes the female is larger than the male; in many Cyprinodonts the male may be only one sixth or even less of the bulk of the female." Likewise, Kyle, in his recent "Biology of Fishes," says of the fresh-water Teleosts, "As a rule the female is larger than the male." If an instance of the condition wherein the male is larger than the female of the same age has been recorded previously for any of the bony fishes, it has escaped the attention of the writers, and seems to have been omitted from Dean's "Bibliography of Fishes."

An analysis of data pertaining to length alone failed to offer definite clue to the separation of the size groups of Table I into age classes. Failure of size increments to give direct evidence of discontinuity between broods of consecutive years seems to find explanation in facts pertaining to the breeding habits of *H. notatus* and to differences in size of the sexes. T. L. Hankinson, in discussing the egg-laying habits of this species in Illinois, states, "The breeding season appears to be a long one; my dates for finding eggs are from May 1 to August 26." This long breeding season would tend to obliterate distinctions in size between the broods of two succeeding years. Individuals hatched early in any given year would have almost as long a favorable growing season as those hatched at the close of the season of the previous year. This observation is supported by the discovery of Hubbs for fishes of northern Michigan, that "the growing season of fishes scarcely extends beyond the four months of June, July, August and September." Though the growing season may be somewhat longer in central Illinois, the restriction of the growing season and a long breeding season combine to obliterate any conspicuous breaks in the sizes of fishes hatched in two consecutive years.

After the sex of all the larger individuals had been determined, a graph was prepared to show the distribution according to sizes of the two sexes in *H. notatus* (Fig. 2). The coincidence of the modes for females and for males in Fig. 2 with the two modes of the graph in Fig. 1 gives conclusive evidence of the correlation between size and sex in this species.

Secondary sexual characters. Only mature males of the blunt-nosed minnow bear characteristic glandular tubercles or pearl-organs on the head. In the material under consideration, these tubercles are not arranged in as regular order as described by Forbes and Richardson for this species. Only the largest males (groups 1 to 3, inclusive, of Table I) have the tubercles fully developed. But seventeen of the thirty-five males measuring 7 cm

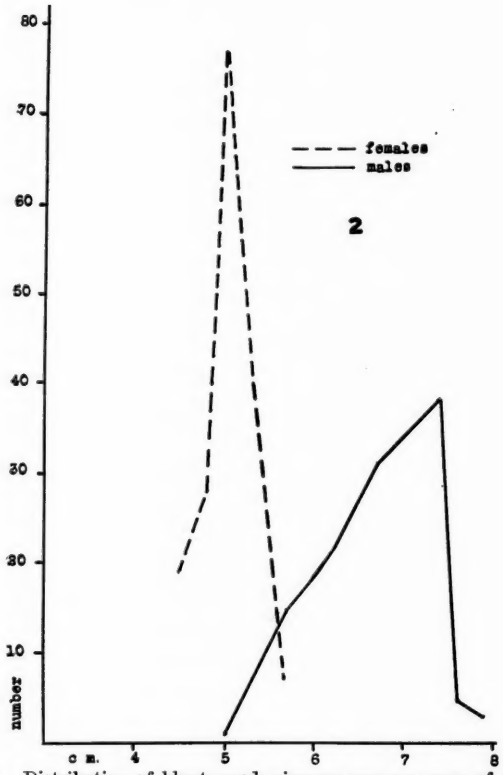

FIG. 2. Distribution of blunt-nosed minnows over one year of age, showing correlations of sex and size. All the females over one year of age (broken line) comprise a single age group just a few months under two years of age. The curve for the males (solid line) is a composite of two age groups and is further analyzed in Fig. 3.

showed these organs, and in these the tubercles were not conspicuous. The remaining eighteen individuals of group 4, though born in the same year as the ones showing tubercles in the early stages of their formation, gave no evidence of tubercle formation, the diagnoses of sex resting wholly upon observation of the gonad.

Observations on the scales. Scales from one or more of each size group mentioned in Table I were removed, freed from epidermis, stained lightly in methylene blue

and mounted in euparal. Under the microscope, the scales of minnows are not so favorable for age determination as are those of sunfishes and many other freshwater species. While the circuli are readily distinguishable, the winter rings or annuli are very indistinct. The latter were recognizable chiefly by virtue of the fact that the circuli become more closely crowded in these regions. The finely graded series of sizes, the relative maturity of the gonads and the large numbers of individuals available for study made it possible to interpret relative and actual age more readily than would have been possible with a smaller number of specimens.

In discussing age determination, reference will be made continuously to the size groups listed in Table I. The scales from all individuals less than 4.5 cm in length (groups 12 to 18 inclusive) show identical scale characters. Since the specimens were killed in April, before the advent of the breeding season of that year, all these smallest individuals had passed but one winter, and on the basis of Hankinson's observations on egg laying in this species, must represent actual ages of from eight to eleven months. For these smallest specimens, each scale has widely spaced circuli at the margin, while adjacent to this marginal region the circuli are more crowded. The wide circuli of the margin represent the period of relatively rapid growth since the opening of spring; the crowded lines represent the retarded growth of the winter just closed.

It is interesting to note that there is a significant break between the size of the largest specimens approaching one year of age and the smallest males in the group approaching their second year. This gap did not become apparent until the sex of the individuals had been determined, for it is bridged by the females which are completing their second year of growth (groups 10 and 11).

As mentioned in an earlier section of this paper, the females in their second year are distinctly smaller than the males of the same age. The median for females near-

ing their second birthday is but 5 cm, while for males of the same age it is 6.2 cm.

No female over two years of age was observed in the material under investigation. The males 7 cm and longer (groups 1 to 4 inclusive) had passed the third winter and were consequently within a few months of three years old (Fig. 3). Since these are the largest males encountered

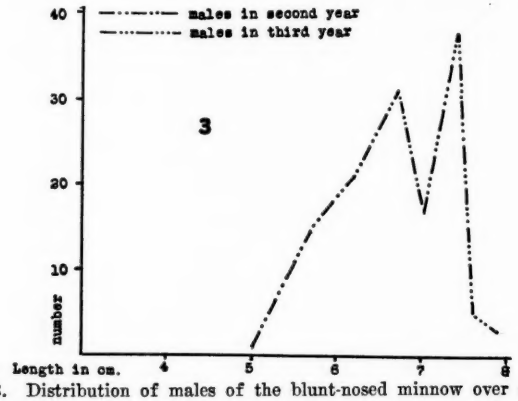

FIG. 3. Distribution of males of the blunt-nosed minnow over one year of age. An analysis of the curve for males shown in Fig. 2, showing size distribution of males approaching the close of their second and third years respectively.

in the thousands of specimens of this species from Champaign County, it seems probable that the male of *H. notatus* becomes sexually mature after his third winter and dies following a single breeding season. The females become sexually mature at two years of age and seem to survive but a single breeding season.

The factors responsible for the differences in age at which the two sexes of the blunt-nosed minnow reach sexual maturity have not been investigated, nor has it been possible to determine why females do not live beyond the second year or males beyond the third.

SUMMARY

1. A single collection of 369 specimens of the blunt-nosed minnow, *Hyborhynchus notatus,* has been sub-

jected to statistical study and microscopical examination for age determination.

2. The specimens were killed in April, before the opening of the breeding season, which other authors have shown to extend in central Illinois from May 1 to August 26.

3. The fishes measured in standard length from 1.3 cm to 7.9 cm.

4. The largest individuals are of the maximum size encountered in a collection of 6,440 specimens of the same species from the same county.

5. Individuals under 4.5 cm are less than one year old with actual ages of from eight to eleven months.

6. In specimens over one year the males are regularly larger than the females: median for males 7 cm. for females 5 cm.

7. Oldest females are slightly under two years of age. Females seem to have a single breeding season and this in the second year.

8. Oldest males are slightly under three years. Only three-year males have pearl organs on the head, and some of smallest males in the three-year age class lack the pearl organs.

LITERATURE CITED

Dean, Bashford
1916–1923. "A Bibliography of Fishes." American Museum of Natural History.

Forbes, S. A., and Richardson, R. E.
1908. "The Fishes of Illinois." Illinois State Laboratory of Natural History, pp. 119, 121.

Günther, A. C. L. G.
1880. "An Introduction to the Study of Fishes." Black, Edinburgh. p. 177.

Hankinson, T. L.
1920. "Notes on the Life-histories of Illinois Fishes." *Trans. Ill. State Acad. Science,* 12: 132–150.

Hubbs, C. L.
1926. "The Structural Consequences of Modifications of the Developmental Rate in Fishes, Considered in Reference to Certain Problems of Evolution," AMERICAN NATURALIST, 60: 57–81.

Kyle, H. M.
1926. "The Biology of Fishes." Macmillan, New York. p. 291.

THE STRENGTH OF ANT MOUNDS

PROFESSOR E. A. ANDREWS
THE JOHNS HOPKINS UNIVERSITY

A COMMON ant of the eastern states, *Formica exsectoides* F., builds earth mounds that serve as nests in which the young are born and reared and to which the food of the entire community is brought home.

As conical masses of earth, knee high to waist high, these nest mounds attract interest as the largest exhibits of communal architecture of the lower animals in this region. Each mound may begin as the work of few ants digging vertical tunnels and depositing the earth between the mounds of the tunnels in irregular heaps for a year or more of working time (half the year being spent in dormancy, down in the earth). But afterwards as the earth pile increases it is excavated within by tortuous branching galleries forming a labyrinth in three dimensions, all roofed over by one continuous layer of earth an inch or less in thickness through which openings from outdoors lead (only about the base of the mound as a rule) into the labyrinth that extends also down into the earth beneath the mound.

Moreover, the original collection of earth excavated from below is eventually added to by collections of vegetable and mineral matter picked up on the surface of the terrain all around about the mound and carried with labor to be deposited upon the mound. This aggregate of two components, the excavated or mined earth and the surface collections, makes the roof, for the time being; periodically the roof is rebuilt.

Observation and experiment indicate that the strength of the roof and the firmness of the materials of the mound in between the cavities of the labyrinth are not the direct products of ant work in the sense that the wax structures of the honey-bee are fabricated after secretion by the bee

or that the paper nest of the hornet is fashioned into shape with secretions of the hornet; rather is the ant mound the result of the continuous work of the ant in collecting materials which the rain wets and thus causes to cohere into more or less resistant mass. That this is the case is indicated by observation of these ants in captivity where within glass tubes plugged with moist clay the ant is seen to cut off with its mandibles minute scrapings of clay then compacted into pellets that the ant may kick out of the way but ultimately transport a short distance and deposit. The mouthfuls or pellets are later used by other ants either to fill in crevices near by or else carried long distances and ultimately distributed, it may be a meter or more from the source, in free spaces within the system of glass tubes simulating a nest. In nature, such pellets are deposited out on the surface of the mound and there collect till rolled off, blown off, held by other débris or ultimately consolidated by water. When such pellets are observed under the microscope and wetted they crumble like sugar in water and make a clay-like mass that when dry is adobe, with considerable coherence and strength.

Communities of these ants kept in the laboratory a year and more both in light and in entirely dark rooms built large mounds from mining and from surface collections; but these mounds remained friable collections of minute particles till consolidated by water artificially applied as a substitute for rain. Then the normal resistant roof resulted.

The strength of the completed mound will thus depend upon three factors: the activities of the ants in collecting materials; the nature of the materials, and the effects of the water and drying.

We have examined both the penetrability and the crushing resistance of various mounds by means of the following crude apparatus: A six-foot cypress stick, one inch square at one end, attached at the other end to a spring scales capable of registering fifty pounds when the

stick is pushed or thrust against the mound with that force, and from that maximum down to about two pounds, the weight of this crude instrument of investigation.

The apparatus tried out on a path did not penetrate at all; in a vegetable garden of clay soil, penetration was only one half to one inch, but in flower garden, with looser soil, penetration was up to two and one half inches for pressure of fifty pounds. The stick breaks through the surface and compresses the soil in proportion as it is loose and weak to resist the pressure.

The mounds, being generally conical, present an apical part and side faces north, south, east and west, and the resistance of the mound may be tried on all these parts. Applied to ant mounds in the region of Baltimore County, elsewhere described,[1] the pressure stick first breaks through the crust or roof of the mound, then penetrates a short distance, then further and yet further with increasing pressures, often stopping and then advancing again as the internal resistances of some buttresses between labyrinths suddenly give way.

Mounds differ much in the character of the soil used in their construction and also furnish very different resistances. In Table I are shown results obtained from examination of sixteen mounds in twenty-six trials.

The left-hand column states the designation of the special mound; the right-hand column its height in inches; the part of the mound tried, whether apex or some special face, is indicated in the second and third columns, while the other columns show the penetration in inches for the pressures shown over each column, the stars standing for mere breaking of the surface, or else unmeasured advance in penetration.

The observations were made in dry weather, on August 9, 1926, except the lowest four, which were made after a week of rain, on August 20, and are put in the table in

[1] E. A. Andrews, "Sequential Distribution of *Formica exsectoides* F.," *Psyche*, 32: 127–150. 1926.

TABLE I

TABLE OF PENETRATION RESISTANCE OF MOUNDS OF *Formica exsectoides* F.

Name of Mound	Part	Face	2	5	10	15	20	25	30	35	40	45	50	Height of Mound
zz							*						2.5	18
jj								*					7.5	18
gg	A		*										9.5	4
iz							*				*		8	29
6					*		*	*					8	27
aa				*	*		*				16			18
X	A			*	4	5		10	14	16	17		17	16
X								4	9				9.5	16
7	A				6		9	9		11.5	14		17.5	22
7								4.5	7.5		9	9	9	22
V		N					2.5	6			8		8	16
V		S						4			7.5		7	24
8						2	3		5				6.5	8
14					3	5		15					17	15
29	A					4	15.5						16	22
29					2			2.5	5		7–11.5		12	22
Beta	A					1		2		6	12		12	7
32	A				6				6.5			8	8.5	34
32		E			1		2						3	34
32		S–W				2.5	3.5		4.5	6			7	34
59	A		*		12	15		18					19.5	36
59		W			2	3.5	7	8.5			10		11	36
Alpha		S			4	5	7	8	10		11	11	12.5	13
Alpha	A			1.5	2.5	4	6–11		13			15	16	13
32		S		1		2.5		4		6			11	34
32	A			1		3	6	13		14	16	16	16	34

order of observation merely. The table indicates that these mounds were more compressible than garden soil, the stick entering 2½ to 19½ inches. Even in the nearly abandoned mound "14" which was partly grass-grown and neglected, the penetration and compression were large for the small height of the mound, indicating a loose interior.

In the larger mounds the penetration with fifty pounds' pressure stops before the level of the earth is reached, but in the little mound "gg," and in the nearly abandoned "14," the stick penetrated beneath the level of the earth, showing that the fabricated or porous structure extended down beneath the earth. This also is found in trials of the apex of "X," of "Beta" and of "Alpha."

In all cases the stick penetrated farther, generally nearly twice as far, when thrust straight down at the apex than when applied at right angles to any of the side faces of the mounds. This is in harmony with observations upon the growth of the mounds, the central parts being younger, more recently built up, less abandoned and more worked over, while the side faces may remain long unopened; the mound growing by a sort of ebullition of the central parts, somewhat comparable to a volcano, with repair of the denudation of lateral parts.

The different sides, N, E, S, W, are not all alike in resistance to pressure, and in some cases this is apparently correlated with age and amount of work done by the ants in fabricating and keeping in repair. The first breaking varies from two to twenty-five pounds and occurs most often at ten pounds. The break through the surface is sudden, as a cracking of a roof and inspection by digging shows a coherent layer of one quarter to one inch deep of dried adobe coming off in fragments as slabs of more or less size according to the coherence of the soil used. The inner face of this roofing is marked by the ceilings of the innumerable tunnels that form a labyrinth throughout much of the mound and penetrate down into the earth

below the mound to some extent. In young mounds the labyrinth is universal throughout the hill, but in very old mounds the lower central part appears largely as mere unused earth and in some mounds only certain faces of the mound are extensively catacombed. When the roof or shell is once broken through the stick advances in proportion as it crushes the walls of the tunnels and fills the voids with earth débris. Where the tunnels are very close together advance is rapid, but where more earth and less void is present the resistance is greater.

In general, the distance penetrated indicates the depth of the part of the mound inhabited, or fabricated so that it may be inhabited by the ants. The exceptional penetration with only two pounds is due to absence of complete roof due to new construction; thus mound "gg" was a very young mound recently started and still in the incipient state of flatness with the surface made up of soft earth in pellets just mined and scattered over the surface; the other mound, "59," was a very ancient mound of large size known to have existed some thirty years, but the ants were working on the apex to increase its height, and thus only loose earth and no roof was present. In such periods of construction there is marked contrast between the old roof and the areas in process of addition to height of mound; a penknife was found to stand up supported by the old roof, but when placed on the recently excavated apical materials it sank in one to one and a half inches.

As far as made out, the solidity of the roof is due to the action of rain in breaking down the pellets of earth and drawing the fine particles together as they subsequently dry off. The last four records in the table show the effects of wet weather. After a week of much rain when the mounds are softened so that the surface yields to the finger like dough sponge, the stick enters more easily and comes out marked with mud. The crust or roof is much weakened, in "Alpha" breaking at the apex with five and

on the south face with ten pounds' pressure, and the penetration extends to or beneath the ground level. In mound "32," the breaking was with five pounds as compared with ten or fifteen when the same mound was dry and the depth reached with fifty pounds was eleven and sixteen inches as compared with three and eight and one half for the same mound when dry. The mounds in this table are constructed of the clay subsoil and sandy top soil in varying proportions with more or less vegetable matter in different mounds.

In Prince George and Montgomery Counties there are mounds built upon light-colored clays that become hard in drying, and some of these mounds on the edges of open fields support the weight of a man with little insinking, being very solid and low elevations of the stiff clay. Five of the mounds resisted the pressure stick as tried in November to the extent shown in Table II, A–E.

A was an old flat remnant of former mound, a mere heap of earth, and was penetrated only one and one half inches with fifty pounds' pressure, which was the same as the neighboring forest floor penetration.

B, a white earth mound, with many pine needles, in grown-up clearing, broke through in middle of south face and at mid apex first with fifteen pounds' pressure.

C, a small white clay mound, sixteen inches in height, in a grown-up field, broke at apex with ten pounds, and was entered with fifty pounds, seventeen inches, thus being soft under the earth level.

D, a mound twenty inches in height, of fine white clay, dry, in a field, did not break on middle of south face till thirty pounds' pressure was applied and then was penetrated nine inches, but the apex broke with ten pounds and was entered two inches. In the middle fifty pounds entered twenty-three inches, thus three inches beneath level of soil.

E, another clay mound in an old field, was moist and this broke at five pounds and was penetrated thirteen inches with fifty pounds.

TABLE II

Mound	Part	Face	Pounds												Height	Circumference
			2	5	10	15	20	25	30	35	40	45	50	60		
A	A												1.5			
B	A					3	10						17			
B		S				*							10			
C					2	9.5							17		16	
D	A				2	13			14				23		20	
D		S							9				14		20	
E				*									13			
1	A		1		7		13		16		11		18		23–26	
1		S			1		6		7		8		9		23–26	
2	A		1		7	12	16		17		18		20		23–26	
2		S		1		4	6		10		15		18		23–26	
3	A			2	5	7		9			13		15		23–26	
3		S		1	2		5	7			9		10		23–26	
4	A		1		4		11	13		16			17		23–26	
4		S		1		7							8		23–26	
5	A		2		6		8		9	13	14		15		23–26	
5		S		1	5	12			15				16		23–26	
6	A		1.5	5.5	8.5		10	11		13			15		23–26	
6		S		1	5		10		12				14		23–26	
I	A									*			14		17	121
I		S					1		1.5				10		17	121
II	A										1		1	17	17	144
II		S								2			11		17	144
III	A						2		3		15				18	216
III		S					1		2	*			9		18	216
IV	A												8		18	108
IV		S							2		8		9		18	108
V	A				1		5			17			17		24	204
V		S					1		2		3	*	15		24	204
VI	A						1	8		13			16		17	120
VI		S					2			5			6		17	120
VII	A						3	5			6	20	21		22	264
VII		S						1		2	11			13	22	264

Thus in Prince George County, the strength of the mounds out in the open where the sun bakes the fine clay is partly due to the fact that the mound presents a stronger roof, breaking often only with fifteen in place of the common ten of the Baltimore County mounds, yet the final penetrability is the same, meaning that the mounds

in both places differ chiefly in the strength of the roofing materials and not in the architecture, which is chiefly a mass of labyrinths hollowed out in the interior of the mound even down to and below the level of the surrounding soil; yet the walls of all the tunnels are much firmer in the stiff clay regions, so that masses of labyrinths can be removed and preserved while in the Baltimore County mounds such fragments generally crumble when not frozen.

Contrasted with these mounds in the open, six mounds in the pine woods were measured in dry weather in May, 1928, with the results shown in Table II as 1 to 6. These six mounds were each from twenty-three to twenty-six inches in height, fine cones with steep sides, made of light gray sandy earth scattered over with more or less abundant pine needles that become ingrown in the process of construction or enlargement of the mounds as permanent components of the mound material.

This table shows that the apex is very much softer than the south face, breaking sooner, with only two pounds of pressure, and being entered fifteen to twenty inches with fifty pounds of pressure, while the south face broke first with five or even ten pounds and was penetrated only eight to eighteen inches. While the pine needles are an element of weakness in the roof they help resist deep penetration when commingled with the soil.

Comparing these dry mounds that have pine needles in their composition with the dry mounds in Table I that had no pine needles, we find that of the seven apex trials of dry mounds in Baltimore County only three broke at two to five pounds, while with the six with pine needles, five broke at two and the other at five; thus these pine mounds were much less strong at the apex, which is in harmony with their sharper, more pointed form due to the needles holding the sand at the steeper angle.

Of the six side face crushings in Baltimore County, three broke at ten, one each at fifteen, twenty, twenty-five,

while the pine mounds broke five at five and one at ten. Here again the pine needle mounds seem much the easier to crush. In general the pine leaf mounds seem to be more easily run through, thus the average penetration for the twelve measurements in Table II is 14.58, while the average for the twenty-two measurements of dry mounds in Table I is 10.36. The former range from eight to twenty; the latter from three to nineteen.

Thus strength of these ant mounds depends upon the character of the soil and upon the fragments of organic matter lying where the ants can make use of them in building; the more mineral the structure, the greater the resistance; the more inwoven or integrated the vegetable matter, the more easy the surface crushing. Moreover, the form of the mound is dependent not only upon living grass, etc., that helps to hold the mineral and thus make steep-sided, round-topped mounds, but upon the character of the long pine needles that help hold the sand in steep, pointed cones. In all cases the surface roof owes its strength to the action of rain, in time solidifying the mineral grains together, while the presence of pine needles, etc., tends to weaken the roof against crushing. The reinforced adobe is less resistant to crushing than is soil not commingled with organic fragments. The essential value of the organic materials collected by the ants and incorporated in the mound is not increase of strength.

The mounds recorded as I–VII in Table II were found a few miles from Oakland, Garrett County, and as indicated they are remarkable for great difficulty of penetration, breaking first at twenty pounds to the square inch. All these mounds, seventeen to twenty-four inches in height, were found near together in a dry glade with sparsely scattered small trees, such as haws, thorns and crabapples, with alders and dense growth of weeds and ferns. Here, as in other counties, some of the mounds were deserted, others in various stages of growth or of

decay, and some were incipient nests. There were not much more than a dozen in all, of which seven were measured and tested for penetrability.

The light clay soil over shale with coal seams bakes in the mounds so hard that a man of 150 pounds can stand without sinking in, on sole of one foot on apex of mound. This extreme strength is shown in the tests indicated in numbers I to VII of trials on the south face and at the apex. It is to be noted that all the left part of the table is here blank, meaning that breaking occurred first with twenty pounds to the square inch in place of the two, five or ten of the Montgomery mounds. This strength seemed due chiefly to the nature of the soil. The mounds were dry and light colored in spite of rain a few days before. However, their strength, especially at the apex, was in part due to the fact that these measurements were taken late in the year, September 23, 1928, when the ants had had a long dry season in which to make complete the repair of the mounds for winter use and there had been no recent excavation work of the ants in adding to the height of the mounds. In fact the ants had withdrawn into winter quarters so completely that very few came out when the test stick penetrated into the mound, and few were seen on digging into the mounds.

In places the roofs of the mounds were as much as one inch thick, of clay with only a few small sticks or grass stems imbedded in its layers. Amongst these mounds were many good illustrations of the tendency of this ant to elongate the mound toward the south; thus No. II measured up the north face one foot, nine inches, but up the south face two feet, nine inches; and No. III was four feet, three inches up the south and only two feet, five inches up the north, while No. VII measured five feet up the south and three up the north.

No. I did not begin to cave in till thirty-five pounds per square inch was applied at the apex. No. II did not break under forty pounds at the apex and needed sixty pounds

to the square inch to penetrate to the bottom of the mound. This great internal strength is in harmony with the observed fact that the labyrinth of these mounds is made in clay that holds firmly so that fragments of the nest might readily be transported as dried clay sponge. The failure of fifty pounds to force down to the bottom of No. IV was partly due to a dense mat of sorrel leaves covering the apex of this deserted mound and offering resistance to the advance of the stick.

The easier penetration of mound V may have been due to rebuilding, as part seemed to have been removed and later restored. Such disturbances in these mounds remote from human interference may be due to bears that eat the ants; also some footprints that penetrate some mounds seemed to result from deer playing about.

The largest mound, VII, like most of the others, had a very hard central core so that with utmost force the stick could not be forced in to the level of the earth, apparently since the oldest part of the mound had reverted to the state of a mere mass of hard clay without the labyrinths that nearer the surface make the fabrication weaker.

In conclusion, these tests of the strength of ant mounds made by the same species of ants in three different parts of the state of Maryland showing marked diversities in surface strengths and in depth of penetration, show that mounds have not only different strengths at different periods of their growth or in different states of moisture, but that there are local diversities dependent upon the character of the soil and materials used by the ants in building the mounds.

The marked increase in strength with use of certain fine clays supports the contention, derived from observation of natural ant activities in the open as well as in specially limited laboratory conditions, that the ant builds chiefly by mining and collecting pellets of earth and vegetable fragments and then depositing them, without consolidating them, so that the mound arises much

as if the particles had been dropped upon it little by little as the sand accumulates in an hour glass. The rain sooner or later leads to physical compacting of the soil into a more or less strong and firm adobe for which the ants have but collected the elements.

That the ants may possibly add some modicum of salivary secretion to some of the earthy materials can not be denied, but there seems here no question of any great utility of secretions in nest-building such as there is in the paper nest of a hornet. Such experiments add a more definite basis for understanding the physical conditions under which these ants exist.

SHORTER ARTICLES AND DISCUSSION

THE EVOLUTION OF DOMINANCE; REPLY TO PROFESSOR SEWALL WRIGHT

In a thoughtful note in the American Naturalist of May–June, 1929, entitled "Fisher's Theory of Dominance," Professor Sewall Wright gives the results of his calculations as to the intensity of the selective agency to which I called attention, and "reaches a figure so 'extremely slow' as to make its efficacy seem highly questionable."

Since Professor Wright has carried out independently some of the calculations given in my original paper (April, 1928), and expresses the results in a different notation, it is perhaps worth while to point out that the numerical values of his primary formulas differ in no essential respects from my own, and that the selective intensity which inclines Professor Wright to reject the theory is in fact the same that originally led me to adopt it.

Professor Wright denotes the frequency of the mutant gene by $1-q$, in place of p; the mutation rate by u in place of k, and the selective disadvantage of the heterozygote by hs in place of $1-v$, and arrives at an equation for equilibrium in the form

$$q = 1 - \frac{u}{hs}$$

in place of the equivalent equation

$$p(1-v) = k$$

of my paper.

At this point a slight divergence occurs between Professor Wright's method and mine, for he proceeds to calculate the intensity of the selection acting upon a single hypothetical modifying factor, whereas my method is to calculate the fraction which the heterozygotes, in which the modifying factors are exposed to selection, will contribute to the ancestry of future generations. Finding that this fraction may on quite reasonable assumptions be as high as one in 5,000, though in other cases it may be much lower, and believing that the number of generations which have occurred in the course of evolution has been more like a million times the number of generations in our experiments, I was led to conclude that this cause of modification could not safely be neglected.

Professor Wright, on the other hand, naturally finds that the intensity of selection is of the same order as the frequency of mutations of the primary factor, and one is only surprised that this fact seems to him to settle the question. That it does so seem is partly due to the fact that Professor Wright has intended to introduce assumptions as favorable as possible to my views. He assumes a single dominant modifier, the effect of which is to cause complete dominance of the wild type gene in the primary factor. The rate of change per generation in the frequency, p' (Professor Wright uses p), of this modifier is approximately calculated, and it is shown that this rate of change, termed "the pressure per generation towards its fixation," is never more than half the mutation rate, k; actually on Professor Wright's formula it never exceeds $8k/27$, which maximum value it attains at $p' = 2/3$.

In case any one should be tempted to quote this result, I ought to point out that it is wrong, in that no account is taken of the effect of the frequency p' of modifying genes on the frequency of the heterozygotes which they modify, and secondly that the rate of change of gene frequency is not a direct measure of the selective intensity, such as Professor Wright requires in the more important difficulty, which he develops later. The rate of change per generation in the gene ratio p' is related to the selective intensity in its favor, which we may call i, by the equation

$$\delta p' = p'(1-p')i,$$

and it is this selective intensity, and not $\delta p'$, which must be compared with any other selective agencies capable of modifying the gene ratio.

In Professor Wright's calculations, the result for selective intensity is that

$$i = 2k(1-p')$$

a quantity which never exceeds twice the mutation rate, k, and decreases progressively as p' is increased. If, using similarly approximate methods, allowance is made for the effect of modifying genes, in increasing the proportion of heterozygotes to be modified, we find

$$i = \frac{2k}{1-p'}$$

which, having the same initial value, increases without limit as p' increases towards unity. The case, of course, is a highly artificial one, and chosen by Professor Wright as especially favorable

to my theory; it is only for this reason that it is worth while showing that a more exact calculation leads to a conclusion so different from that drawn by him. I do not in the least wish to dispute that the selective intensity will be proportional to, and generally of the order of, the mutation rate, k, though the fact that the evolution of dominance by selection proceeds with increasing speed as dominance becomes more complete is an essential point stressed in my original note.

The real difficulty, however, felt by Professor Wright is not so simple as that the selective action upon dominance modifiers is so small that there has not been time for it to have had any appreciable effect. He makes no mention of the time probably available for these changes, in the course of evolution, and agrees with me as to the probability of demonstrating the efficacy of such selection experimentally. Where he really differs from me is in my assumption that a small selective intensity of say 1/50,000 the magnitude of a larger one will produce the same effect in 50,000 times the time. He suggests that the gene ratio of the modifying factors will either be held in stable equilibrium by more powerful forces, so that a minute selective intensity will merely shift to a minute extent the position of equilibrium, and produce no progressive effect—as if the complex of gene ratios were a *gel* rather than a *sol*—or be irresistibly increased or decreased by selective agencies so powerful that a minute additional selective intensity can only delay or accelerate the extinction of the less favored gene, without ever being able to determine which gene shall be extinguished—much as a wind blowing along a railroad will not exert any effect in accumulating rolling stock at the leeward terminal.

This is a criticism genuinely aimed at my theory; I do make the assumption, and the assumption really may be doubted. It is a well-directed criticism, but not a very strong one. Professor Wright can not say, "Certain observational facts do seem to support your theory, but your conjectural cause is demonstrably too feeble to have brought about its supposed effects," unless he adds, "provided I am right in my own conjecture that small selective intensities do not have effects proportional to their magnitude." My theory, in fact, is opposed, not by an obstinate fact of arithmetic, but by a rather original conjecture as to the inefficacy of minute selections, or as to the agencies controlling the gene ratios of modifying factors.

There seems little probability of direct evidence upon so subtle a question. I have myself attached a good deal of importance, in other connections, to gene ratios held in stable equilibrium, though it never occurred to me that such a state was universal. Even if it were nearly so, the conditions on which the stability rests must be liable to change; stability may turn to instability, a transient state, admittedly, but one in which a minute, but steadily increasing, selective intensity would be well fitted to tip the balance. As to the ratios having neutral stability, there is one reason for thinking that the factors suffering the feeblest selective action will at any one time be the most numerous. The fate of those powerfully selected is quickly settled; they do not long contribute to the variance. It is the idlers that make the crowd, and very slight attractions may determine their drift. On the whole, it seems that the most reasonable assumption which we can make, on an obscure subject, is that the effect is approximately proportional to the cause.

Any progressive change, which is very slow in its action, may be represented, for the sake of paradox, as dependent from very rare, and therefore very improbable events. In the course of time the height of a mountain is lowered by 1,000 feet; at one instant its height is above 10,000 feet, a second later it is below. What an improbable event! That the topmost grain should just protrude a fraction of a millimeter above 10,000 feet, and then that some purely conjectural meteorological accident should remove it. How can any one take seriously such an extravagant series of assumptions? Equally improbable seems the extinction or "fixation" of modifying genes, a very rare, but occasionally necessary, consequence of the slight constant tendencies at work in modifying their frequencies.

R. A. FISHER

ROTHAMSTED EXPERIMENTAL STATION,
HARPENDEN, ENGLAND

THE EVOLUTION OF DOMINANCE

COMMENT ON DR. FISHER'S REPLY

DR. FISHER'S objection to my statement[1] that the pressure per generation toward fixation of a modifier of dominance "is never more than half that which would result from recurrent mutation from m to M at the same rate as that for A to a" is undoubtedly

[1] AMER. NAT., 1929, 63: 274–279.

well taken. If account be taken of the effect of the frequency of the modifier on the frequency of the heterozygote, which must be done if p is to depart much from the value 1, my formula for the relative reproductive rates of the individuals with and without the modifier becomes $1:1-2up^2$ instead of $1:1-2u$. Similarly, the change in the value of p per generation (selection pressure) becomes $\delta p = 2u\ (1-p)$ (until p is nearly 0, when δp itself rapidly approaches 0). This agrees with Fisher's result for i "selection intensity" defined as $\frac{\delta p}{p\ (1-p)}$.

It still appears to me, however, to be proper to compare $\delta p = 2u\ (1-p)$, the change in frequency due to the selection, with $\delta p = up$, the change in frequency in the same direction due to mutation at rate u; or better, with the change due to opposing mutation at this rate, which is $u\ (1-p)$. In the latter case, the selection pressure, being always just twice the mutation pressure (until dominance is nearly complete), would in time practically fix the modifier against such mutation. But, as Dr. Fisher notes, the case was chosen as the most favorable to the theory which could be expected in nature. The selection pressure on a gene which merely increases the dominance of another by a small fraction becomes a third order phenomenon, if direct selection and mutation be considered as of the first and second order respectively. Dr. Fisher stresses increasing speed of selection as dominance becomes more complete but the speed can never reach twice the mutation rate of the mutant, unless the frequency of the mutant heterozygote is made greater than its value at equilibrium. The latter remains rather low ($\sqrt{\frac{u}{s}}$) even when dominance is complete, because of the selection against the homozygous mutant. In the laboratory one can readily pick out the heterozygotes for breeding, *i.e.*, practice artificial selection *against* wild type, and thereby enormously increase the natural selection in favor of heterozygotes which *resemble* the wild type, but such a process of opposing selections would seem to have no counterpart in nature.

As there seems to be no essential disagreement on this matter of rate, we may come to the real point at issue: can a selection pressure of this order produce any appreciable evolutionary effect, however long it may continue? There seems to be no disagreement on the view that the fate of a factor is determined

by the net effect of the opposing evolutionary forces and that practically this is determined by only the most important of these. Dr. Fisher's comparison of the influence of a second order factor with that of a continuous wind in controlling the destination of a railroad train expresses well the idea. The question at issue reduces then to whether there are genes so neutral in relation to all other evolutionary forces that selections which change their frequencies at rates of the order of mutation pressure are the most important forces acting on them, and whether such genes are sufficiently numerous to give a basis for such a common phenomenon as dominance. Dr. Fisher holds on the whole to the affirmative and I am still skeptical, admitting that questions are involved on which we know very little.

I have not raised the question of insufficiency of geologic time, to which Dr. Fisher refers, although a factor which at best requires a number of generations greater than the reciprocal of the natural mutation rate per locus probably has little to spare in this respect.

My first point was that, in the majority of cases, the selection rate of Fisher's hypothesis would be unable to fix the modifier even against an average mutation pressure. With mutation acting in the same direction as selection, the gene would be fixed but the selection would merely be playing the rôle of a following wind in the case of the railroad train.

It seemed unlikely to me, however, that mutation itself is a factor of appreciable importance in fixing genes. I raised the probability that all genes have multiple effects and, through one or other of these, each in general is subject to direct selection which takes precedence in controlling its fate. The recent estimates of the total number of genes in Drosophila as only a few thousand are suggestive in this connection.

I do not hold, however, that even the most important selective action on a gene is necessarily the controlling factor. This brings up considerations relating to the stability of gene frequencies, which I did not discuss in my previous paper but which are raised by Dr. Fisher as favorable to his view. Both of us used the conditions of equilibrium in connection with the ordinary mutations whose characteristic recessiveness constitutes the problem. Such factors are in general rare in nature, and probably always have been rare. Though continually mutating down the ages, they are kept from rising appreciably above the esti-

mated figure for equilibrium by powerful negative selection. The equilibrium may be considered stable for the present purpose. On the other hand, factors which are almost neutral to all other evolutionary forces should be highly unstable with respect to gene frequency. I can not accept the view that this instability is favorable to the success of feeble selection. In fact, I would say that it sets a lower limit below which selection is not effective.

In a freely interbreeding population of limited size (n) gene frequency shows random variation with a standard deviation $\sqrt{\frac{q(1-q)}{2n}}$. Being random, such variations largely neutralize each other, but there is a second order drift which can not be ignored. Dr. Fisher himself[2] has calculated the probability distribution resulting from this cause under various conditions. Thus with no selection and very low mutation rate, he found the distribution $df = \frac{1}{2\pi} \operatorname{sech} \frac{1}{2} z dz$, where $z = \log \frac{q}{1-q}$. Translated into terms of q, this becomes $\frac{1}{\pi} q^{-\frac{1}{2}} (1-q)^{-\frac{1}{2}} dq$, which is a U shaped distribution. The factor is usually close to fixation one way or the other but may drift from one extreme to the other. There can hardly be said to be any equilibrium point. He showed that feeble selection does not alter the situation in this respect. I have made a similar calculation by a different method and have reached a general formula (unpublished) which agrees roughly with Dr. Fisher's results. I find the probability of different values of q to be proportional to $e^{2nsq} q^{4nv-1} (1-q)^{4nu-1}$, where s measures the selection favoring the gene whose frequency is q, u the mutation rate of this gene and v that of reverse mutation. The approximation is quantitatively valid only for weak selections and does not, of course, give the frequencies of the terminal, temporarily fixed classes, which are $\frac{f_1}{4nv}$ and $\frac{f_{2n-1}}{4nu}$ respectively, where f_1 and f_{2n-1} are the frequencies of the subterminal classes as given approximately by the formula. The form of the curve under different conditions indicates whether selection (s), isolation effect $(\frac{1}{2n})$, or mutation (u, v) is the factor which controls the fate of the gene and thus in a sense adjudicates between the principles of Darwin, Wagner and de Vries, respectively.

[2] *Proc.* Roy. Soc. Edinburgh, 1922.

Selection controls the situation if s is larger than $\frac{1}{2n}$ but is of little importance below this figure. In small inbred populations ($\frac{1}{2n}$ large) even vigorous selection is ineffective in keeping injurious factors from drifting into fixation, and in the histories of a number of such lines one can follow in the laboratory the course of evolution under extreme isolation. In the case of Fisher's modifiers of dominance with selection coefficients at best of the order of mutation rate, the latter must be greater than $\frac{1}{2n}$ if the gene is not to drift back and forth in the course of geologic time from one state of approximate fixation to the other and practically as freely in the face of the selection pressure as with it.

Unfortunately it is difficult to estimate n in animal and plant populations. In the calculations, it refers to a population breeding at random, a condition not realized in natural species as wholes. In most cases random interbreeding is more or less restricted to small localities. These and other conditions such as violent seasonal oscillation in numbers may well reduce n to moderate size, which for the present purpose may be taken as anything less than a million. If mutation rate is of the order of one in a million per locus, an interbreeding group of less than a million can show little effect of selection of the type which Dr. Fisher postulates even though there be no more important selection process and time be unlimited.

The non-adaptive nature of the differences which usually seem to characterize local races, subspecies and even species of the same genus indicates that this factor of isolation is in fact of first importance in the evolutionary origin of such groups, a point on which field naturalists (*e.g.*, Wagner, Gulick, Jordan, Kellogg, Osborn and Crampton) have long insisted. With nearly complete local isolation and correspondingly large value of $\frac{1}{2n}$, one might expect to find the entire probability array of values of q exhibited in different localities contemporaneously in the case of factor pairs of little selective significance. It seems likely then that, in many cases, factors which are not fixed by rather strong selection will be subject to this effect and thus irre-

sponsive to feeble selection of the order of effectiveness of mutation pressure.

Both Dr. Fisher and I have dealt only with the case of pairs of allelomorphs. If mutations are occurring in many directions in each gene, as seems likely, a complete analysis would require consideration of an indefinitely extended multiple allelomorphic series. This complicates the matter, but it seems to me that here also those genes which are not controlled by moderately strong selections would ordinarily drift at random through the multiple dimensional system of gene frequencies, regardless of any second or third order evolutionary pressures, and that consequently the explanation of the frequency of dominance as a Mendelian phenomenon must be sought elsewhere. The suggestion that mutations most frequently represent inactivations of genes, and that, for simple physiological reasons, inactivation should generally behave as recessive, still seems adequate as a positive alternative hypothesis.

SEWALL WRIGHT

UNIVERSITY OF CHICAGO

A DOMINANT WHITE FLOWER COLOR IN *BRASSICA OLERACEA* L.

THE flower petals of *Brassica oleracea* are usually yellow, occasionally white (Bailey, '22). *B. alboglabra* Bailey is an Asiatic form having white flowers. Sinskaia ('27) describes it as closely related to *B. oleracea,* intercrossing readily. Karpechenko ('28) reports that in the unpublished data of a colleague, N. N. Timofeyoff, the white petal character has been found to be dominant. This is, as far as I know, the only reference to the inheritance of this character.

In a field of Jersey kale grown near Berkeley, California, in 1926, several plants were observed to bear white flowers. Pollen from one of these plants was applied to castrated flowers of a plant of St. Valentine broccoli; an excellent set of seeds was secured. Forty-three F_1 plants were grown at the University Farm, Davis, California, in 1926–27; twenty-five of these had white flowers, eighteen yellow. In 1928–29 more F_1 plants of this cross were grown; twenty-seven of these had white flowers, thirty-two yellow. Thus, of this cross, 26×01, fifty-two plants had a white and fifty had a yellow corolla, a ratio of nearly 1:1. All the progeny of the maternal plant had yellow petals; the

paternal plant was in a commercial field; no selfed seed was obtained from it, therefore its genetic composition is unknown. It was apparently heterozygous for this character.

F_2 populations were grown from several F_1 plants, both yellow and white petalled, in 1927–28 at Davis. F_1 plants with yellow

TABLE I

SEGREGATION FOR COROLLA COLOR IN A CROSS OF YELLOW-FLOWERED BROCCOLI AND WHITE-FLOWERED KALE

		F_2 phenotypes			Selections				F_3 phenotypes		
Line	F_1 phenotype	White	Yellow	Ratio	Plant no.	Phenotype	Plants grown	Plants blooming	White	Yellow	Ratio
27175	Yellow	4*	15								
27176	"	0	15		7	Yellow	13	12	0	12	
27180	"	2*	62								
27181	"	0	28								
27184	"	0	44								
27188	"	0	32								
27189	"	0	38		2	Yellow	16	5	0	5	
					22	"	32	31	0	31	
					30	"	3	2	0	2	
27190	"	0	41								
27201	"	2*	70		38	"	43	24	0	24	
27202	"	3*	33								
27269	"	0	103								
27177	White	64	27	2.81 – 1.19							
27178	"	6	4	2.40 – 1.60							
27179	"	17	8	2.72 – 1.28	3	White	12	6	6	0	
					13	"	10	4	4	0	
					15	"	10	4	2	2	2.0 – 2.0
					16	"	5	5	4	1	3.2 – 0.8
27182	"	22	7	3.03 – 0.97							
27183	"	15	6	2.86 – 1.14							
27185	"	23	10	2.78 – 1.22	17	"	24	8	8	0	
27186	"	6	5	2.18 – 1.82							
27270	"	43	17	2.87 – 1.13	1	"	44	40	33	7	3.3 – 0.7
		196	84								
Expected 3:1		210	70								
Deviation			14								
Probable error			4.89								

Probability of occurrence per 100 trials, 5.39.

* Plants considered the result of contamination.

petals produced progeny with yellow petals except in a few instances where the ratio was such that it was obvious that contamination had occurred. All the lines derived from white petalled F_1 plants segregated for white and yellow color, in the proportion of approximately three white to one yellow, as shown in Table I. The probable errors for Tables I and II were taken

TABLE II

SEGREGATION IN THE BACKCROSS POPULATIONS

			Backcross phenotypes					
		Parents	Color of corolla	White corolla	Yellow corolla	Ratio	Deviation	Probable error
28 × 087	♀	5–1–4	Yellow	36	34	1.03 : 0.97	1.0	2.82
	♂	27270–1	White					
28 × 089	♀	5–1–7	Yellow	22	21	1.02 : 0.98	0.5	2.21
	♂	27270–1	White					
28 × 090	♀	27270–1	White	14	14	1.00 : 1.00	0.0	1.78
	♂	5–1–7	Yellow					
28 × 115	♀	5–1–4	Yellow	5	5	1.00 : 1.00	0.0	1.07
	♂	27270–5	White					
28 × 115b	♀	5–1–2	Yellow	10	10	1.00 : 1.00	0.0	1.51
	♂	27270–5	White					
28 × 121	♀	27270–14	White	16	0	2.00 : 0.00	.....	.....
	♂	49–1	Yellow					
28 × 122	♀	27270–5	White	8	13	0.77 : 1.23	2.5	1.54
	♂	49–1	Yellow					
26 × 01	♀	2–6	Yellow	52	50	1.02 : 0.98	1.0	3.41
	♂	8	White					

from tables calculated by the department of plant breeding at Cornell University, and the probability of occurrence per one hundred trials from Pearl and Miner ('14). The deviation is rather large and since contamination occurred in some lines, it is possible that it occurred in other lines also. Although the flowers were protected from visitation by larger insects, no provision was

made to exclude thrips, which Annand ('26) found to carry pollen. In later work the mouths of the paper sacks have been lined with cotton, which gives an insect-tight union with the raceme.

In 1928–29 several F_3 and backcross populations were grown. The segregation of the F_3 populations is shown in Table I and of the backcrosses in Table II. A number of other F_3 populations were planted out, but because of an unavoidable delay in transplanting to the field in the autumn, together with an unfavorable winter season, they did not flower and were discarded.

The F_3 populations are very small and not all the plants in some lines produced flowers, so that the data on the segregation are very limited. However, yellow-flowered parents produced only yellow-flowered progeny. These populations are sufficiently large to establish this fact. The F_3 data on the segregations for white and yellow are very unsatisfactory, since the populations that produced only white-petalled plants were not large enough to prove definitely that they were homozygous for this factor, and the ratios in the two populations that segregated for white and yellow are not conclusive. However, the data from the backcrosses are much more conclusive, the ratios being very close to the expected, even the largest deviation being less than twice the probable error. One of the F_2 plants, 27270–14, appeared to be homozygous for the factor for white petal.

From the data presented it is evident that a white corolla in *Brassica oleracea* L. is conditioned by one dominant factor, for which I suggest the symbol "Wh." The petals of nearly all cultivated Brassicas are yellow, the only exceptions of which I know being *B. alboglabra* Bailey, a variety of sprouting broccoli from Italy, and these few Jersey kale plants found in a commercial planting in California. A dominant character of such scattered occurrence may either be closely linked with some undesirable commercial character which would eliminate it from seedsmen's stocks through selection, or it may be a relatively recent mutation. Since dominant mutations are relatively rare, further studies are in progress to determine its linkage relations.

O. H. PEARSON

DIVISION OF TRUCK CROPS,
UNIVERSITY OF CALIFORNIA,
DAVIS

LITERATURE CITED

Annand, P. N.
1926. "Thysanoptera and the Pollination of Flowers." AMER. NAT., 60: 177–182.

Bailey, L. H.
1922. "Gentes Herbarium," Vol. I. Fasc. II.
Karpechenko, G. D.
1928. "Polyploid Hybrids of *Raphanus sativus* L. × *Brassica oleracea* L." *Zeit. f. Ind. Abst. und Vererbungslehre,* 48: 1–85, pl. 3.
Pearl, R. and H. R. Miner.
1914. "Table for Estimating the Probable Significance of Statistical Constants." *Maine Agr. Exp. Sta. Bul.,* 226: 85–89.
Sinskaia, E. N.
1927. "Geno-systematical Investigations of Cultivated Brassicas." *Bul. Appl. Bot. and Pl. Breeding,* 17: 141–166.

INDICATIONS OF A FLAGELLATE STAGE IN *EUGLYPHA ALVEOLATA*

Shell State

On April 14, 1925, there appeared in one of my old protozoan cultures at Columbia University some *Euglypha alveolata.* I picked up five individual *Euglyphae* with a capillary pipette under low power microscope, with as few bacteria as possible, and put them into previously sterilized 250 cc flasks containing Knop's solution and plugged with cotton.[1] About five sterile cover glasses were placed in the bottom of each flask. The flasks were then placed aside to incubate until July 9. The bacteria seemed to break down the Knop's solution and feed upon it. *Euglypha* then fed upon the bacteria. By this method shell forms only were obtained; these had long anastomosing pseudopodia which caught numerous bacteria for food (Figs. 1, A), causing the alveolar zone to become filled with food vacuoles. The three regions described by Schewiakoff (3) were less prominent as a result of this method of feeding. I made only one transfer between April 14, 1925, and December 5, 1926. In order to study the organism the cover glasses were removed with sterile inoculating needles, wiped on the under side and examined under the microscope.

On August 1, 1927, *Euglyphae* were taken out of the Knop's solution (2) and put into tobacco infusion plus bacteria. The mitochondria became less distinct and ceased making shell plates. The organism then left the shell in an amoeba-like stage (Figs. 6, 7, F, G, H).

[1] Trans. Amer. Micro. Bio., 1928.

AMOEBOID STAGE

The ectosarc could hardly be distinguished from the endosarc, as the amoeba was very sluggish in its movements (Figs. 6, 7, F, G, H). Cytoplasmic fusions occur in both the shell and amoeboid forms. Fig. 2 shows two shell forms with their cytoplasm fused. The significance of this is explained by Blochmann (1) and I have observed a similar phenomenon in the amoeboid stage (Figs. 7, 8, E, J, K). These amoeba fused together after having undergone division. I have counted as many as eight amoebae, in a row with their cytoplasm fused. In the process of fusion the cytoplasm of one organism flows over to the other. The chromatin material is given off by the nucleus of the smaller amoeba, until the cytoplasm is filled with chromatin. This may occur before fusion (Fig. F) the chromidia then passing over with the cytoplasm to the larger amoeba where they seem to fuse with the nucleus (Fig. E). The old degenerate nucleus is cast off (Figs. 8, J, K). Following the fusion of the cytoplasm the amoeba undergoes successive division for sixteen days, becoming smaller at each division (Figs. 9, 10). The mitochondria can still be seen but not as distinctly.

The three zones described by Schewiakoff (3) in the shell forms can still be made out. The flagellum now extends out from the characteristic nucleus which was at the attached end in the shell stage (Figs. 11, 12, M, L, P, Q, S).

FLAGELLATE STAGE

The flagellum originates in the karyosome and pushes its way out of the nucleus into the cytoplasm, pointing in the direction of the posterior end of the animal (Figs. H, L). The chromatin material which comes from the karyosome (Fig. 11) seems to be surrounded by a thick cytoplasmic sheath, which is often pushed out far beyond the posterior end, like a handle on a bell (Figs. 11, S). The flagellum then extends from this sheath. The flagellum is always connected with the karyosome until the basal body is formed (Fig. M). In Figs. 11, 12, A, and S mitrochondria can still be seen surrounding the nucleus.

The nucleus divides by promitosis (Figs. 12, M).

The animal moves very slowly and the lashing of the flagellum is slow and at irregular intervals.

When food becomes scarce the flagellum is withdrawn and all indigestible material is thrown off. The animal rounds itself

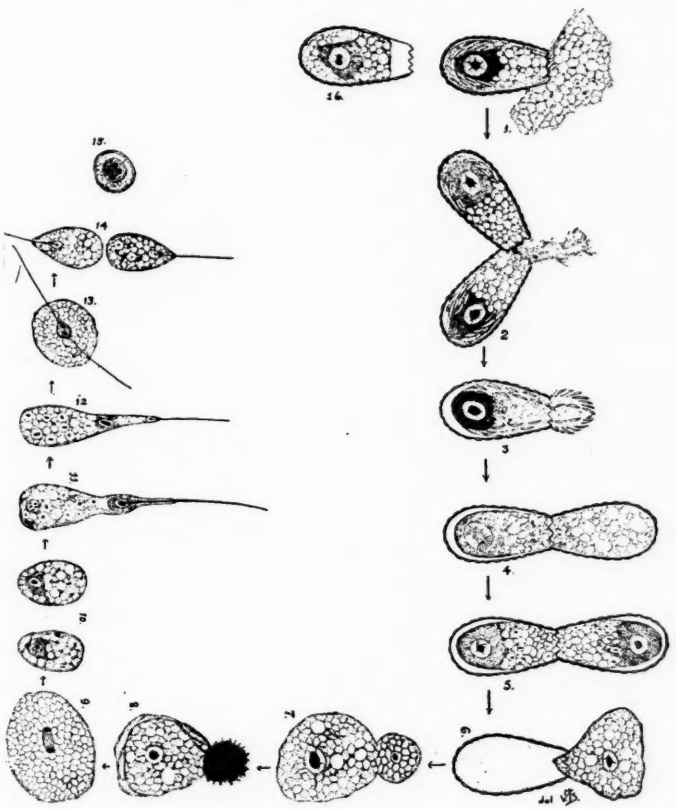

PLATE I.

EXPLANATION OF FIGURES

FIG. 1. Shell form with extended pseudopodia feeding on bacteria.

FIG. 2. *Euglypha* with their cytoplasm fused. The cytoplasm of one will pass over to the other, leaving the old degenerate nucleus behind.

FIG. 3. Shell stage, showing the development of the daughter cell. The protoplasm is flowing out of the mother cell to form the daughter cell.

FIG. 4. Nucleus in anaphase, the daughter cell larger than the mother cell.

FIG. 5. The nucleus has divided and passed to the posterior end of each shell.

FIG. 6. Contents leaving the shell as an amoeba.

FIG. 7. The cytoplasmic fusion of two amoebae.

FIG. 8. The old degenerate nucleus of one is thrown off.
FIG. 9. Amoeba undergoing rapid division.
FIG. 10. Small amoebæ after rapid division.
FIG. 11. Flagellum arising from karyosome. Notice the flagellum is surrounded by a thick cytoplasmic sheath which is pushed out far beyond the posterior end. Mitochondria still surrounding the nucleus.
FIG. 12. The nucleus undergoing division by promitosis without withdrawing the flagellum.
FIG. 13. A flagellate undergoing division while rounded up. The karyosome has divided and a flagellum extends from each.
FIG. 14. Two flagellates which arose as a result of division.
FIG. 15. Cyst of flagellates.
FIG. 16. Cyst of the shell forms.

PLATE II

FIG. A. Shell form with extended pseudopodia feeding on bacteria.
FIG. B. Shell stage, showing the development of the daughter cell. The nucleus moves up towards the orifice of mother cell preparing for division, while the protoplasm is flowing out of the mother cell to form the daughter cell.
FIG. C. *Euglypha* encysted. Protoplasm drawn into the posterior end of shell. Mitochondria drawn around nucleus, plotoplasm becoming dense, vacuoles have disappeared.
FIG. D. Amoeba after rapid division, the mitochondria still surrounds the nucleus.
FIG. E. The fusion of the amoeba. The chromidia pass over with the cytoplasm and fuse with the cytoplasm of the other amoeba.
FIG. F. Amoeba after leaving the shell, the chromidia being distributed throughout the cytoplasm just before fusion.
FIG. G. Amoeba after leaving the shell, the nucleus preparing for division.
FIG. H. Amoeba feeding. The ectosarcs can hardly be distinguished from endosares.
FIGS. J, K. After the chromidia and cytoplasm of one amoeba have fused with those of the other the old degenerate nucleus is given off.
FIG. L. Flagellate stage showing the flagellum connected with the centrosome.
FIGS. M, N, O. The nucleus undergoing division before the blepharoplast divides. The flagellum extending out from the blepharoplast.
FIGS. P, Q, S, T. Flagellates taking in bacteria. The three zones described by Schewiakoff are distinct.
FIG. R. Flagellate rounded up; the flagella and blepharoplast have divided.
FIG. U. Flagellate cyst. The nucleus breaking up into chromatin.

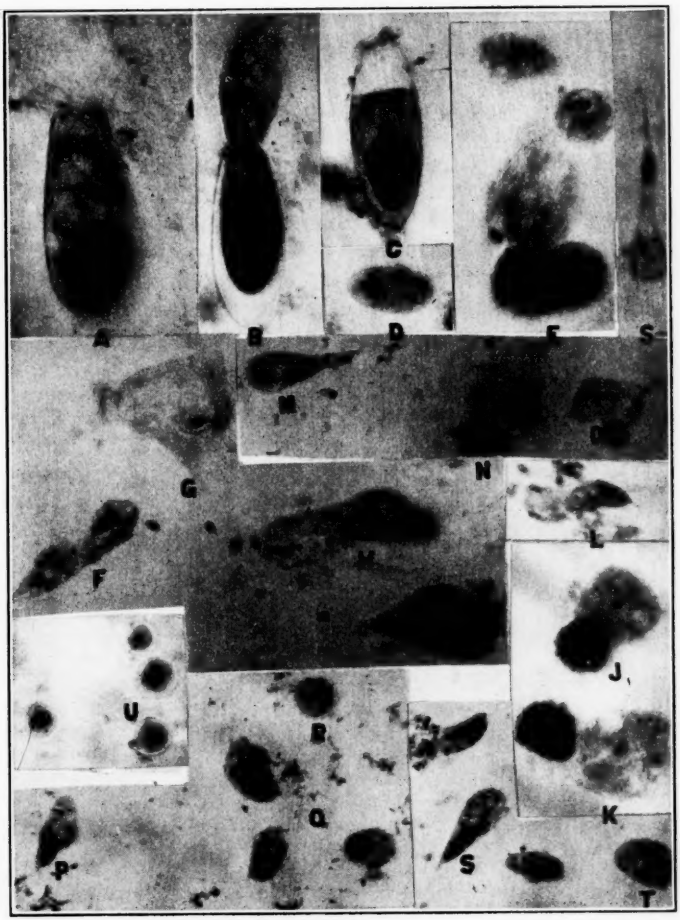

PLATE II.

up with the nucleus in the center, then secretes a cell wall about itself (Figs. 15, U). I have tried repeatedly to get them to excyst but without success. After a week's time the karyosome fragments resemble the fragmented karyosome of the shell cyst.

CONCLUSIONS

Having followed *Euglypha alveolata* in different media through the shell, amoeboid and flagellate forms and having observed therein its changes in morphology, such as mitochondria, development of shell plates, nuclear division, development of flagella and encystation, I feel that I have sufficient reason to conclude that I have been dealing with the same animals throughout. I realize that there is a good chance for contamination in keeping a culture going for five years and I doubly regret therefore that I could not get the cysts to excyst, as I had hoped to carry them back to the shell forms and so complete the cycle.

PHILIP M. JONES

UNIVERSITY OF MINNESOTA

LITERATURE CITED

(1) Blochmann, F. "Zür Kenntnis der Fortpflanzung Von *Euglypha alveolata*, Duj. Morph. Jahrbuch XIII, pp. 173–183, 1888.

(2) Jones, P. M. "Mitochondria and Their Secretions during Cell Division of *Euglypha aveolata*," Trans. Amer. Micro. Bio., 1928.

(3) Schewiakoff, W. "Über die karyokinetische Kuntheiling der *Euglypha alveolata*," Morph. Jahrbuch XIII, pp. 215–245, 1888.

SEX REVERSAL IN *PANDALUS DANAE*[1]

DURING the summer of 1928, while working at the Biological Station, Nanaimo, on the rate of growth of *Pandalus danae* Stimpson, the common commercial shrimp of British Columbia, I measured some hundreds of shrimps and noted the sex. Although there were large numbers of small males, no females were taken smaller than 6 cm and only one smaller than 7 cm. The lengths of males indicated that they were in two year-groups, one averaging about 6 cm, the other about 8 cm. On the other hand the females seemed to belong to a third year-group with an average length of about 10 cm. This appeared to suggest that the form under consideration was protandrous, and a closer study was undertaken.

In the males the secondary sexual characters consist of an organ of copulation on the inner ramus of the first pleopod, and an appendix masculina in addition to the usual appendix interna at the base of the inner ramus of the second pleopod. Both are lacking in the female. Moreover the inner ramus of the first pleopod in the male is comparatively rounded at the tip, while in the female it is drawn out to a point. Specimens smaller than 3.5 cm do not show the male secondary sexual characters, nor have their first pleopods the shape characteristic of females, hence they are evidently merely immature.

More careful observation disclosed several specimens, varying in length from 7 cm to 9 cm, that had intergrading secondary sexual characters. That is to say, the organ of copulation on the first pleopod and the appendix masculina on the second were found in various stages of degeneration, and the tip of the first inner ramus was gradually becoming more drawn out. Dissection of these forms showed an ovary, apparently quite normal, but with two pairs of ducts. The anterior pair, the oviducts, passed straight to the bases of the third pereiopods; while the posterior pair, which still showed the characteristic winding structure of vasa deferentia, went to the bases of the fifth pereiopods.

Dissections were then made of females at various stages of egg production, and in all there were remnants of vasa deferentia, though in the older specimens these traces were often little but a slightly twisted thread. It could not be determined with certainty whether any sign of oviducts appeared in the males.

[1] This work was carried out with the aid of a scholarship from the National Research Council.

Examination of males showed oval depressions, containing thinner chitin than the surrounding integument, in the same positions on the third pereiopods as the openings of the oviducts in the female. Similarly the otherwise definite females have oval depressions at the bases of the fifth pereiopods corresponding to the openings of the vasa deferentia in males. The extra openings, however, do not appear to be functional in either males or females.

Histological examination of the gonads supports the ideas suggested by the external appearance. Serial sections were cut of the gonads from several stages of males and from a female. In the younger males the testis was filled with developing sperms, except for a small part in the center of each half which contained several young egg cells. These last were much larger cells than the spermatogonia, and quite typical in structure. In a fully mature male the sperms had all passed into the vasa deferentia which were enormously distended, while the gonad was in a very collapsed condition. The egg cells were somewhat enlarged, darkly staining granules were beginning to appear in the cytoplasm, and developing oogonia were to be seen at one side. In the female, at a stage when the eggs were still far from ripe, no trace of sperms could be found in the ovary. The male stage was only represented by a few small remnants of the vasa deferentia, which also contained no sperms. On the other hand, the egg cells had multiplied to a considerable extent. The older cells had been forced to the periphery and their cytoplasm was full of darkly staining granules, while developing oocytes were still to be found in the center.

As stated above, a study of the lengths shows two main year-classes for the males and one for the females. The evidence, then, seems to indicate that *Pandalus danae* functions as a male during its second year, and as a female during its third.

As far as I have been able to ascertain, this is the most complete case of change of sex yet reported for Decapoda. Instances have, however, been noted under the heading of "hermaphroditism" for *Calocaris macandreae* (Runnström, 1925), *Lysmata seticaudata* (Spitschakoff, 1912), three species of *Gebia* (Ishikava, 1891, and Runnström, 1925) and three species of *Parastacus* (Lönnberg, 1898, and Runnström, 1925). The first two examples are certainly more truly hermaphroditic than *Pandalus danae,* but a closer study may well show both *Gebia*

and *Parastacus* to undergo a sex reversal very similar to that of *Pandalus.*

Specimens have been found showing degenerating male secondary sexual characters in several of the other species of Pandalids taken in British Columbia. It therefore seems reasonable to suppose that these forms also show similar sex reversal.

ALFREDA A. BERKELEY

DEPARTMENT OF BIOLOGY,
UNIVERSITY OF TORONTO

LITERATURE CITED

C. Ishikava. "On the Formation of Eggs in the Testis of *Gebia major*, de Haan." *Zool. Anz.*, 14. 1891.

E. Lönnberg. "Some Biological and Anatomical Facts concerning Parastacus." *Zool. Anz.*, 21. 1898.

S. Runnström. "Beitrag zur Kenntnis einiger hermaphroditischen Dekapoden Crustacean." *Bergens Mus. Skrift.* N. F. 3, no. 2. 1925.

Th. Spitschakoff. "*Lysmata seticaudata*, Risso, als Beispeil eines echten Hermaphroditismus bei den Decapoden." *Zs. wiss. Zool.* Leipzig, 100. 1912.

NUMBER OF MAMMAE IN MUSKRATS

IT has been previously supposed that the number of mammae in the muskrat is six.[1] However, from a number of observations on two species and three subspecies of the genus *Ondatra*, I find the number to range from eight to eleven.

Thirteen study skins of adult females of *Ondatra zibethica zibethica* from Michigan and Massachusetts and two adult females of *Ondatra zibethica cinnamomina* from Iowa and Kansas, in the mammal collection of the Museum of Zoology at the University of Michigan, were examined, as well as an adult female *Ondatra zibethica osoyoosensis* from eastern Utah in the author's collection.

The table shows that in the specimens of this species examined, the number ranged from eight to eleven and that most of the specimens had more than eight.

In 1926, I reported on the number of mammae of the muskrat in Louisiana (*Ondatra rivalicia*)[2] as judged from pelts taken by trappers.

[1] Hollister, "Systematic Synopsis of the Muskrats," "North American Fauna," vol. 32, 1911, page 14.

[2] *Seventh Biennial Report* of the Department of Conservation of the State of Louisiana, 1924–26, page 80.

Mammae	Pectoral		Inguinal		Number of skins
	Right	Left	Right	Left	
Ondatra zibethica zibethica					
11	3	4	2	2	1
10	3	3	2	2	5
9	3	2	2	2	4
9	2	3	2	2	1
8	2	2	2	2	2
Ondatra zibethica cinnamomina					
10	3	3	2	2	2
Ondatra zibethica osoyoosensis					
11	3	4	2	2	1

Out of 537 pelts examined, 213 were females. Of these 213 females, 177 had eight mammae, 2–2 pectoral 2–2 inguinal; thirty-two had nine mammae, 3–2 pectoral 2–2 inguinal; three had ten mammae, 3–3 pectoral 2–2 inguinal; one had nine mammae, 2–2 pectoral 3–2 inguinal. These pelts were average lots containing "kits" (young muskrats), mediums and adults. There were several cases where "kits" had nine mammae.

This would indicate that the young muskrats were born with the "extra" mamma, assuming that eight is the more usual number.

Comparing the number of mammae within the two species, *Ondatra zibethica* shows a greater variation (assuming that the group of specimens examined was a true sample) than does *Ondatra rivalicia*. In the former, fourteen out of sixteen skins had more than eight mammae, while in the latter only thirty-six out of 177, or approximately one fifth of the specimens, had more than eight mammae. From this evidence it seems that the average number of mammae may vary with the species.

The pectoral region in both species was the area of greatest variation. The number of mammae in the inguinal region remained almost constant.

There is every indication that all mammae were functional in the adult, because of their size and evidence of lactation.

It is interesting to note that when more than two mammae are present on a side, the "extra" mammae are found in between the more usual two, so that the milk line is not lengthened. This was the case both in the pectoral and inguinal regions.

ARTHUR SVIHLA

MUSEUM OF ZOOLOGY,
UNIVERSITY OF MICHIGAN

INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS

Alabama argillacea, The Mystery of, G. N. WOLCOTT, 82
ALLARD, H. A., Cleaning Habits of a Katydid, 283
ALPATOV, W. W., and R. PEARL, Duration of Life, 37
Amebas Mayorella bigemma and M. (?) dofleini, A. A. SCHAEFFER, 88
ANDREWS, E. A., Ant Mounds, 540
Autonomous Organs, Tube Feet of Starfishes as, V. L. PAINE, 517

Balanus crenatus Bruguiere, Attachment of, R. M. BOHART, 353
BEADLE, G. W., Chlorophyll Deficiency in Maize, 189
BEERS, C. D., Reproduction in the Ciliate Didinium without Conjugation or Endomixis, 125
BERKELEY, A. A., Sex Reversal in Pandalus danae, 571
Birds as Hosts for the Common Chigger, H. E. EWING, 94
BOHART, R. M., Attachment of Balanus crenatus Bruguiere, 353
BONNIER, G., The Quantitative Theory of Sex, 186
BRINK, R. A., and C. R. BURNHAM, Semi-sterility in Maize, 301
BROWN, L. A., Cladocerans and Temperature, 248, 346, 443

CHIDESTER, F. E., and W. M. INSKO, JR., Influence of Desiccated Thyroid and Iodine on Growth, 239
Chlorophyll Deficiency in Maize, G. W. BEADLE, 189
Chromosome Counts in Vitis and Pyrus, B. R. NEBEL, 188
Ciliary Movement, J. GRAY, 68
Cladocerans and Temperature, L. A. BROWN, 248, 346, 443
CLAUSEN, R. E., and W. E. LAMMERTS, Hybridization in Nicotiana, 279
CLELAND, R. E., and F. OEHLKERS, Genetical Peculiarities in the Oenotheras, 497
COCKERELL, T. D. A., Hybrid Sunflowers, 470
CONKLIN, E. G., Problems of Development, 5
Correlation Coefficients, J. A. HARRIS, 181

DAWSON, J. A., and W. H. MITCHELL, JR., Viability of Infusorian Cysts, 476
Development, E. G. CONKLIN, 5
Dominance, Fisher's Theory of, S. WRIGHT, 274; of Bar over Infra-Bar in Drosophila, S. WRIGHT, 479; Evolution of, R. A. FISHER, 553; S. WRIGHT, 556
Dominant, Mutations of the Japanese Morning Glory, Y. IMAI and K. TABUCHI, 362; White Flower Color in Brassica oleracea, O. H. PEARSON, 561
Drosophila, melanogaster, Somatic Genovariations in, N. W. TIMOFEEFF-RESSOVSKY, 118; Changes in Gene Alignment produced by X-Rays, H. J. MULLER and T. S. PAINTER, 193; Effect of the X-Chromosome upon Bar-Eye in, A. H. HERSH, 378; Translocation in, H. J. MULLER, 481; Duration of Effects of X-Rays on Male Germ Cells in, F. B. HANSON and F. HEYS, 511

EMERSON, R. A., Hybrids of Perennial Teosinte and Maize, 289
EWING, H. E., Birds as Hosts for the Common Chigger, 94

Fibrillar System in Protozoa, Function of, C. V. TAYLOR, 328
FISHER, R. A., Evolution of Dominance, 553
Fishes, Poisonous, D. S. JORDAN, 382
Flagellate Stage in Euglypha alveolata, P. M. JONES, 565

GRAY, J., Ciliary Movement, 68
Growth, Influence of Desiccated Thyroid and Iodine on, F. E. CHIDESTER, and W. M. INSKO, JR., 239

HANSON, F. B., and F. HEYS, Radium and Lethal Mutations in Drosophila, 201; Duration of Effects of X-Rays on Male Germ Cells in Drosophila melanogaster, 511
HARRIS, J. A., Correlation Coefficients, 181
HAYES, H. K., and H. E. BREWBAKER, Linkage Studies of Factor Pairs in Maize, 229
Hermaphroditism, J. H. LLOYD, 130
HERSH, A. H., The X-Chromosome and Bar Eye in Drosophila melanogaster, 378
Hibernation of the Ground Squirrel, G. E. JOHNSON, 171
Hybrid Sunflowers, T. D. A. COCKERELL, 470
Hybridization in Nicotiana, R. E. CLAUSEN and W. E. LAMMERTS, 279
Hybrids of Perennial Teosinte and Maize, R. A. EMERSON, 289

IMAI, Y., Segregation of Albescent Seedlings, 151; and K. TABUCHI, Dominant Mutations of the Japanese Morning Glory, 362

JOHNSON, G. E., Hibernation of the Ground Squirrel, 171
JONES, P. M., A Flagellate Stage in Euglypha alveolata, 565
JORDAN, D. S., Poisonous Fishes, 382

Katydid, Cleaning Habits of a, H. A. ALLARD, 283

Life, Duration of, W. W. ALPATOV and R. PEARL, 37
LINDSTROM, E. W., Linkage in Maize, 317
Linkage, Studies of Factor Pairs in Maize, H. K. HAYES and H. E. BREWBAKER, 229; E. W. LINDSTROM, 317
LLOYD, J. H., Hermaphroditism, 130
Lobsters, Larval and Postlarval, D. A. MACKAY, 160
LORD, E. M., and W. H. GATES, New Mutation of the House Mouse, 435

MACKAY, D. A., Larval and Postlarval Lobsters, 160
Mammae, Number of, in Muskrats, A. SVIHLA, 573
MANGELSDORF, P. C., Length of Styles and Mendelian Segregation in Maize, 139
METZ, C. W., "Unisexual" Progenies in Sciara, 214; Sex Determination in Sciara, 487
Minnow, Blunt-nosed, H. J. VAN CLEAVE and H. C. MARKUS, 530
MULLER, H. J., Translocation in Drosophila, 481; and T. S. PAINTER, Changes in Gene Alignment Produced by X-Rays in Drosophila, 193
Mutation of the House Mouse, E. M. LORD and W. H. GATES, 435
Mutations, Lethal, in Drosophila, F. B. HANSON and F. HEYS, 201; A. S. SEREBROVSKY, 374

NEBEL, B. R., Chromosome Counts in Vitis and Pyrus, 188
Neurofibrils, G. H. PARKER, 97

Oenotheras, Genetical Peculiarities in, R. E. CLELAND and F. OEHLKERS, 497

PAINE, V. L., Tube Feet of Starfishes as Autonomous Organs, 517
PARKER, G. H., Neurofibrils, 97; The Loggerhead Turtle, 367
PATERSON, D. G., and E. G. WILLIAMSON, Raymond Pearl on the Doctrine of "Like Produces Like," 265
Pearl, Raymond, on the Doctrine of "Like Produces Like," D. G. PATERSON and E. G. WILLIAMSON, 265
PEARSON, O. H., A Dominant White Flower Color in Brassica oleracea L., 561
PETRUNKEVITCH, A., Spider Fauna of Panama, 455
Protoplasm, Contractility of, W. SEIFRIZ, 410

Reproduction, Indefinite, Possibility of, C. D. BEERS, 125
RIDDLE, O., Thyroid Races in Ring Doves, 385

SCHAEFFER, A. A., Amebas Mayorella bigemma and M. (?) dofleini, 88
Sciara, "Unisexual" Progenies in, C. W. METZ, 214; Sex Determination in, C. W. METZ, 487
Segregation, Mendelian, in Maize, P. C. MANGELSDORF, 139; of Albescent Seedlings, Y. IMAI, 151
SEIFRIZ, W., Contractility of Protoplasm, 410
SEREBROVSKY, A. S., Mutations, 374
Sex, The Quantitative Theory of, G. BONNIER, 186; Determination in Sciara, C. W. METZ, 487; Reversal in Pandalus danae, A. A. BERKELEY, 571
Shorter Articles and Discussion, 88, 188, 274, 374, 470, 553
Spider Fauna of Panama, A. PETRUNKEVITCH, 455
Sterility, Semi, in Maize, R. A. BRINK and C. R. BURNHAM, 301
SVIHLA, A., Number of Mammae in Muskrats, 573

TAYLOR, C. V., Function of the Fibrillar System in Protozoa, 328
Thyroid Races in Ring Doves, O. RIDDLE, 385
TIMOFEEFF-RESSOVSKY, N. W., X-Rays and Somatic Genovariation in Drosophila melanogaster, 118
Turtle, Loggerhead, G. H. PARKER, 367

VAN CLEAVE, H. J., and H. C. MARKUS, The Blunt-nosed Minnow, 530
Viability of Infusorian Cysts, J. A. DAWSON and W. H. MITCHELL, JR., 476

WOLCOTT, G. N., Alabama argillacea, 82
WRIGHT, S., Fisher's Theory of Dominance, 274; Dominance of Bar over Infra-Bar in Drosophila, 479; Evolution of Dominance, 556